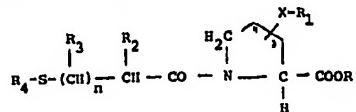

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(54) Mercaptoacyl derivatives of substituted prolines

(57) New mercaptoacyl derivatives of substituted prolines which have the general formula



or salts thereof may be used as hypotensive agents.

In the formula X-R₁ group is located at the 3- or 4-position of the proline ring;

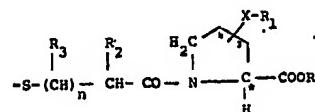
X is oxygen or sulfur;

R is hydrogen or lower alkyl;

R₁ is lower alkyl, lower alkenyl, lower alkynyl, phenyl, substituted phenyl, phenyl-lower alkylene, or substituted phenyl-lower alkylene;

R₂ and R₃ are independently selected from hydrogen, lower alkyl and trifluoromethyl;

R₄ is hydrogen, R₅-C(=O)- or



R₅ is lower alkyl, phenyl, or phenyl-lower alkylene; and n is 0, 1 or 2.

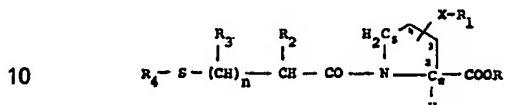
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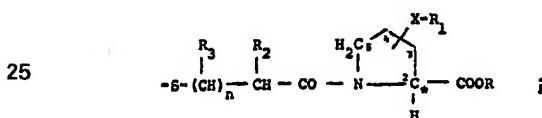
SPECIFICATION**Mercaptoacyl derivatives of substituted prolines**

- 5 This invention provides new ether and thioether mercaptoacyl prolines of the formula (I)



wherein the group X - R₁ is located at the 3- or 4-position in the ring;

- 15 X is oxygen or sulfur;
R is hydrogen or lower alkyl;
R₁ is lower alkyl, lower alkenyl, lower alkynyl, phenyl, substituted phenyl, phenyl-lower alkylene or substituted phenyl-lower alkylene;
20 R₂ and R₃ are independently selected from hydrogen, lower alkyl, and trifluoromethyl;
R₄ is hydrogen, R₅ - CO - or



- R₅ is lower alkyl, phenyl, or phenyl-lower alkylene; n is 0, 1 or 2;

30 and salts thereof.

The invention in its broadest aspects relates to the ether and thioether mercaptoacyl prolines having formula I above and salts thereof, to compositions containing such compounds and to the method for using such compounds as anti-hypertensive agents.

The term lower alkyl as used in defining the symbols R, R₁, R₂ and R₃ are straight or branched chain hydrocarbon radicals having up to seven carbons, for example, methyl, ethyl, propyl, isopropyl, butyl, 40 isobutyl, t-butyl, pentyl, isopentyl, etc. The preferred lower alkyl groups are up to four carbons with methyl and ethyl being most preferred.

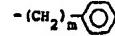
The term lower alkenyl as used in defining the symbol R, are mono-unsaturated straight or 45 branched chain hydrocarbon groups of from 2 to 7 carbons such as ethenyl, propenyl, isopropenyl, butenyl, and the like. The lower alkynyl groups are straight or branched chain hydrocarbon groups of from 2 to 7 carbons having one triple bond, e.g., 50 propargyl. The preferred lower alkenyl groups are from 2 to 5 carbons and the preferred lower alkynyl groups are from 3 to 4 carbon atoms.

The term substituted phenyl and substituted phenyl-lower alkylene as used in defining the symbol R, include one or two, preferably one, substituents on the phenyl ring. Suitable substituents include lower alkyl groups of 1 to 4 carbons, especially methyl, lower alkoxy groups of 1 to 4 carbons, especially methoxy, lower alkylthio groups of 1 to 4 55 carbons, especially methylthio, halogens, especially chloro or fluoro, trifluoromethyl, acetoxy, and hydroxy. The hydroxy substituted phenyl and phenyl-

lower alkynes are obtained by hydrolysis of the corresponding acetoxy substituted phenyl compound as the last step of the synthetic procedure.

The term halogen includes the four common members, i.e., chloro, bromo, fluoro, and iodo, with chloro, bromo, and fluoro being preferred.

The term phenyl-lower alkylene as used in defining the symbols R₁ and R₅ include groups such as



- 75 wherein m is an integer from 1 to 4. Preferred phenyl-lower alkylene groups are phenylmethyl and phenylethyl, especially phenylmethyl.

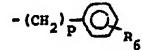
The lower alkanoyl groups represented by R₅ - CO - are those having the acyl radicals of the lower (C₂ - C₇) fatty acids, for example, acetyl, propionyl, 80 butyryl, isobutyryl, and the like. The lower alkanoyl groups having up to four carbons are preferred with acetyl being especially preferred. Similarly, when R₅ in the group R₅ - CO - is phenyl-lower alkylene, benzoyl is especially preferred.

85 The asterisk in formula I indicates an asymmetric center which is present in the proline ring. Of course, an additional asymmetric center can be present in the mercapto sidechain depending upon the substituents R₂ and R₃. The products of formula I accordingly exist in stereoisomeric forms or as racemic mixtures thereof. All of these are within the scope of the invention. The synthesis described below can utilize the racemate or one of the enantiomers as starting materials. When the racemic starting material is used in the synthesis procedure, the

90 stereoisomers obtained in the final product can be separated by conventional chromatographic or fractional crystallization methods. The X - R₁ group also gives rise to cis-trans isomerism.

95 100 Preferably the asymmetric center in the proline ring is in the L-configuration and if there is an asymmetric center in the mercaptoacyl sidechain it is in the D-configuration.

Preferred compounds of formula I are those 105 wherein R is hydrogen; R₁ is lower alkyl of 1 to 3 carbons or



- 110 wherein p is zero, 1 or 2, and R₆ is hydrogen, methyl, methoxy, methylthio, chloro, fluoro, trifluoromethyl or hydroxy; R₂ is hydrogen, methyl or trifluoromethyl; R₃ is hydrogen; n is zero or one; and R₄ is hydrogen. Also preferred as intermediates are the

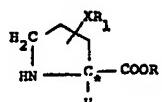
115 above compounds wherein R₄ is acetyl or benzoyl, especially acetyl.

Most preferred are the above compounds wherein X is oxygen; R₁ is methyl or ethyl, especially methyl; n is 1; R₂ is hydrogen or methyl, especially methyl;

120 R₃ is hydrogen; R₄ is hydrogen; and the -XR₁ group is at the 4-position of the proline ring, especially wherein the -XR₁ group is in the cis configuration.

The ethers and thioethers of Formula I are obtained by coupling the ether or thioether proline

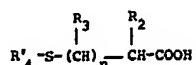
of the formula (II)



5

with the acid or its chemical equivalent of the formula (III)

10



wherein R'4 is hydrogen or R5 - CO -, to yield the product of the formula (IV)

20

This reaction can be effected in the presence of a coupling agent like dicyclohexylcarbodiimide or the like, or the acid can be activated by formation of its mixed anhydride, symmetrical anhydride, acid halide, active ester or use of Woodward reagent K, N - ethoxycarbonyl - 2 - ethoxy - 1,2 - dihydroquinoline or the like. For a review of the methods of acylation, see Methoden der Organischen Chemie (Houben-Weyl), Vol. XV, part II, page 1 et seq. (1974). Preferably, the acid halide, especially the acid chloride, of formula III is reacted with the acid of formula II.

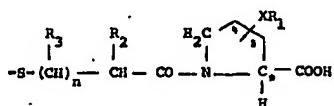
The ester compounds of formula IV, i.e., R is alkyl, can be converted to the free acid, i.e., R is hydrogen, by conventional means. For example, if R is t-butyl treatment with trifluoroacetic acid and anisole gives the free acid.

The product of formula IV is preferably isolated and purified by crystallization, e.g., by forming the dicyclohexylamine salt and then converting the salt to the free acid form by treatment with an aqueous solution of an acid, such as potassium acid sulfate.

The product of formula IV bearing the acyl group R5 - CO - can be converted to the products of formula I wherein R4 is hydrogen by conventional hydrolysis or by ammonolysis.

The products of formula I wherein R4 is

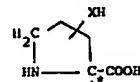
50



are obtained by directly oxidizing with iodine a product of formula I wherein R4 is hydrogen.

The esters of formula I wherein R is lower alkyl can be obtained from the carboxylic acid compounds, i.e., wherein R is hydrogen, by conventional esterification procedures, e.g., by esterification with a diazoalkane such as diazomethane, 1 - alkyl - 3 - p - tolyltriazene, such as 1 - n - butyl - 3 - p - tolyltriazene, or the like.

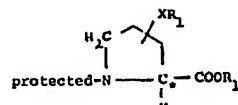
The proline reactants of formula II can be prepared by various means. For example, a hydroxy or mercapto proline of the formula



70 is acylated with an acylating agent such as acetic anhydride, acetyl chloride, propionic anhydride, butyric anhydride, benzylchloroformate, or the like, so as to protect the nitrogen. The R1 group is then introduced by reacting the N-protected form of the

75 compound of formula V with a halide, R-hal, wherein hal represents a halogen, preferably iodine, in the presence of silver oxide, sodium hydride, sodium hydroxide or the like, to obtain the intermediate having the formula (VI)

80

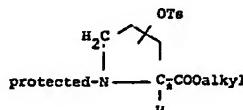


85

Alkaline hydrolysis of the intermediate of formula VI with a base such as barium hydroxide, sodium hydroxide, potassium hydroxide or the like first yields the free acid (COOH) and then hydrolysis with a mineral acid, such as sulfuric acid, yields the starting material of formula II.

Another method for preparing the proline reactants of formula II is by treating the N-protected tosylxy proline ester, preferably the methyl ester of the formula (VII)

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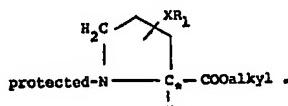


with the sodium salt of the formula (VIII)



105 to yield the intermediate of the formula (IX)

110



In formula VII the symbol Ts represents $-SO_2-\text{C}_6\text{H}_4-\text{CH}_3$

and the N-protecting group is benzyloxycarbonyl, 115 which is preferred, or other commonly employed acyl protecting groups. In this reaction, if the tosylate group is in the cis configuration the XR1 group will be in the trans configuration and if the tosylxy group is in the trans configuration the XR1 group will be in the cis configuration. The intermediate of formula IX is then treated to remove the alkyl ester group and is then reacted with hydrogen bromide to yield the HBr salt of the proline reactant of formula II which can then be coupled with the acid, preferably the

120 acid chloride, of formula III.

The proline starting materials of formula II wherein X is sulfur and the XR1 group is attached at the 3-position of the proline can also be obtained by treating a 1,2 - dehydroproline ester, preferably the

130 t-butyl ester, of the formula (X)



with an acylating agent such as benzyl chloroformate, acetyl chloride, etc., to yield the 4,5 - dehydro compound (XI)



10 which is then reacted with the mercaptan R₁ - SH to yield (XII)



15 wherein the - S - R₁ substituent is in the trans configuration. The N-protecting and alkyl groups are then removed to yield the desired starting material.

20 The proline starting materials of formula II where R₁ is phenyl, substituted phenyl, phenyl-lower alkylene or substituted phenyl-lower alkylene can also be obtained by treating the benzyl ester of the N-protected proline of formula V with the alcohol R₁ - OH in the presence of triphenylphosphine and diethylazodicarboxylate according to the procedure of Bittner et al., Chemistry and Industry, 15 March 1975, page 281. Removal of the N-protecting group and the benzyl ester group yields the starting material of formula II.

Reference is also made to the following publications for additional illustrative information with respect to the production of starting materials and intermediates: Ondetti et al., U.S. Patents 4,046,889, 35 4,105,776, and 4,154,935; Neuberger, J. Chem. Soc., 1945, p. 429-432; Patchett et al., J. Amer. Chem. Soc. 79, p. 185-192 (1957); Baer et al., Can. J. Biochem. and Phys., 37, p. 583-587 (1959); Sheehan et al., J. Amer. Chem. Soc. 85, p. 3863-3865 (1963); 40 Magerlein, J. Med. Chem. 10, p. 1161-1163 (1967). The procedures illustrated therein can be utilized as general methods for the synthesis and stereoconversion of compounds utilizable in the invention of this application.

45 Additional experimental details are found in the examples which are preferred embodiments and also serve as models for the preparation of other members of the group.

The compounds of this invention form basic salts 50 with a variety of inorganic or organic bases. The salt forming ion derived from such bases can be metal ions, e.g., aluminum, alkali metal ions, such as sodium or potassium, alkaline earth metal ions such as calcium or magnesium, or an amine salt ion, of 55 which a number are known for this purpose, for example, aralkylamines such as dibenzylamine, N,N - dibenzylethylenediamine, lower alkylamines such as methylamine, t - butylamine, procaine, lower alkylpiperidines such as N - ethylpiperidine, cycloalkylamines such as cyclohexylamine or dicyclohexylamine, 1 - adamantanamine, benzathine, or salts derived from amino acids such as arginine, lysine or the like. The physiologically acceptable salts such as the sodium or potassium salts can be used medically 60 as described below and are preferred. These 65

and other salts which are not necessarily physiologically acceptable are useful in isolating or purifying a product acceptable for the purposes described below, as illustrated with the dicyclohexylamine salt

- 70 and the cyclohexylamine salt in the examples. The salts are produced by reacting the acid form of the compound with an equivalent of the base supplying the desired basic ion in a medium in which the salt precipitates or in aqueous medium and then 75 lyophilizing. The free acid form can be obtained from the salt by conventional neutralization techniques, e.g., with potassium bisulfate, hydrochloric acid, etc.
- The compounds of this invention inhibit the conversion of the decapeptide angiotensin I to angiotensin II and therefore may be used in reducing or relieving hypertension. The compounds of this invention intervene in the renin → angiotensinogen → angiotensin I → angiotensin II sequence by inhibiting angiotensin converting enzyme and reducing or 80 eliminating the formation of the pressor substance angiotensin II. Thus by administration of a hypotensively effective amount of a composition containing one or a combination of compounds of formula I or physiologically acceptable salt thereof (together 85 with a pharmaceutical carrier), hypertension in the species of mammal suffering therefrom is reduced or alleviated.

A single dose, or preferably two to four divided daily doses, provided in a basis of about 0.1 to about 95 100 mg. per kilogram per day, preferably about 1 to about 50 mg. per kilogram per day, is appropriate to reduce blood pressure as indicated in the animal model experiments described by S. L. Engel, T. R. Schaeffer, M. H. Waugh and B. Rubin, Proc. Soc. Exp. Biol. Med. 143, 483 (1973). The substance is preferably administered orally, but parenteral routes such as subcutaneously, intramuscularly, intravenously or intraperitoneally can also be employed.

The compounds of this invention can be utilized to 105 achieve the reduction of blood pressure by formulating in compositions such as tablets, capsules or elixirs for oral administration or in sterile solutions or suspensions for parenteral administration. About 10 to about 500 mg. of a compound or mixture of compositions of formula I or physiologically acceptable salt is compounded with a physiologically acceptable vehicle, carrier, excipient, binder, preservative, stabilizer, flavor, etc., in a unit dosage form as called for by accepted pharmaceutical practice. The 110 amount of active substance in these compositions or preparations is such that suitable dosage in the range indicated is obtained.

Illustrative of the adjuvants which may be incorporated in tablets, capsules and the like are the following: 115 a binder such as gum tragacanth, acacia, corn starch or gelatin; an excipient such as dicalcium phosphate or microcrystalline cellulose; a disintegrating agent such as corn starch, potato starch, alginic acid and the like; a lubricant such as magnesium stearate; a sweetening agent such as sucrose, lactose or saccharin; a flavoring agent such as peppermint, oil of wintergreen or cherry. When the dosage unit form is a capsule, it may contain in addition to materials of the above type a liquid carrier 120 such as a fatty oil. Various other materials may be 130

present as coatings or to otherwise modify the physical form of the dosage unit. For instance, tablets may be coated with shellac, sugar or both. A syrup or elixir may contain the active compound, sucrose 5 as a sweetening agent, methyl and propyl parabens as preservatives, a dye and a flavoring such as cherry or orange flavor.

Sterile compositions for injection can be formulated according to conventional pharmaceutical 10 practice by dissolving or suspending the active substance in a vehicle such as water for injection, a naturally occurring vegetable oil like sesame oil, coconut oil, peanut oil, cottonseed oil, etc.

The following examples are illustrative of the 15 invention and constitute preferred embodiments. They also serve as models for the preparation of other members of the group which can be produced by replacement of the given reactants with suitably substituted analogs. All temperatures are in degrees 20 Celsius.

Example 1

1 - (3 - Acetylthio - 1 - oxopropyl) - trans - 4 - methoxy - L - proline
a) N - Acetyl - trans - 4 - hydroxy - L - proline
25 A stirred suspension of 26.2 g. (0.2 mole) of trans - 4 - hydroxy - L - proline in 400 ml. of acetic acid is treated with 26 ml. of acetic anhydride. The solid gradually dissolves after 2 hours of stirring at room temperature. The solution is transferred to a 2 liter 30 flask and the solvent is removed on a rotary evaporator at a bath temperature of 45°. The syrupy residue (57.5 g.) is diluted with 100 ml. of ether to give a crystalline solid. After cooling overnight, the solid is filtered, washed with cold ether and dried in 35 a desiccator. This material (35.7 g.) is pulverised and suspended in 100 ml. of ether, cooled and filtered to give 33.8 g. (98%) of N - acetyl - trans - 4 - hydroxy - L - proline, 128-131°. Recrystallization of 0.5 g. of this material from 5 ml. of acetonitrile gives 0.45 g. of 40 colourless solid, m.p. 130-132°; $[\alpha]^{25}_D -92^\circ$ (c, 1% in EtOH).
b) N - Acetyl - trans - 4 - methoxy - L - proline, methyl ester

A mixture of 30.0 g. (0.17 mole) of N - acetyl - trans 45 - 4 - hydroxy - L - proline and 130 g. of silver oxide is pulverized in a mortar and this intimate mixture is added to a 1-liter flask with 300 ml. of acetone. The slurry is stirred, treated portionwise with 130 ml. of methyl iodide and the temperature maintained 50 below 40° by cooling with a cold water bath. After stirring for 7 hours, the mixture is allowed to stand overnight. The solid is filtered, washed well with acetone and the filtrate concentrated on a rotary evaporator to give 38.3 g. of syrupy residue. The latter is redissolved in 350 ml. of acetone and again treated with 130 g. of silver oxide and 130 ml. of methyl iodide to give 41 g. of residue. The latter is distilled to yield 32.2 g. of distillate; b.p. 130-140° (0.3 mm). After digestion in 30 ml. of cyclohexane and 55 cooling, the nearly colorless solid N - acetyl - trans - 4 - methoxy - L - proline methyl ester weighs 31.4 g., m.p. 71-75°. Recrystallization from 31 ml. of ethyl acetate gives 25.1 g. (66%) of colorless solid, m.p. 76-77°. $[\alpha]^{25}_D -83^\circ$ (c, 1% in EtOH).

65 c) trans - 4 - Methoxy - L - proline

To a stirred solution of 27.0 g. (0.085 mole) of barium hydroxide · 8H₂O in 525 ml. of water (approx. 3.3 N) is added 11.0 g. (0.05 mole) of N - acetyl - trans - 4 - methoxy - L - proline methyl ester. 70 The resulting solution is stirred at 18-20° for 3 hours, cooled and treated with dilute sulfuric acid (8.8 g. of conc. H₂SO₄ in 20 ml. of water). The acidic suspension is allowed to stand overnight. The mixture is filtered through a thick layer of Celite to give a "milky" filtrate. The latter is concentrated on a rotary evaporator at 50° using a high vacuum pump to give a milky residue weighing 121 g. This material is treated with dilute sulfuric acid (19.0 g. of conc. H₂SO₄ in 75 ml. of water) and the resulting mixture is stirred 80 and refluxed for 3 hours. After cooling to 30°, the mixture is treated portionwise with 48 g. of barium hydroxide · 8H₂O and the pH then adjusted from 6.0 to 4.0 with dilute sulfuric acid. After standing overnight, the mixture is filtered through a thick layer of Celite. The "milky" filtrate is concentrated as above to give 50 g. of colorless dry residue. The latter is digested with 200 ml. of hot chloroform and filtered through a bed of Celite to remove the barium sulfate. The slightly turbid filtrate is concentrated on a rotary 85 evaporator to give a gelatinous material (17.7 g.), suspended in 100 ml. of ether, and filtered to give 7.5 g. (94%) of nearly colorless solid, m.p. 185-190° (dec.). This material is suspended in 30 ml. of warm acetonitrile, cooled and filtered to give 4.0 g. (50%) of 90 a colorless solid, trans - 4 - methoxy - L - proline, m.p. 209-211° (dec.); $[\alpha]^{25}_D -75^\circ$ (c, 1% in EtOH).
d) 1 - (3 - Acetylthio - 1 - oxopropyl) - trans - 4 - methoxy - L - proline
A solution of 3.5 g. (0.024 mole) of trans - 4 - 100 methoxy - L - proline in 50 ml. of water is stirred, cooled to 5° and 3 g. of sodium carbonate are added. This mixture is treated with a solution of 4.0 g. (0.024 mole) of 3 - acetylthiopropionyl chloride in 5 ml. of ether during the course of 10 minutes with the 105 intermittent addition of 3 g. of sodium carbonate to maintain the pH at about 8.0. The mixture is stirred in the ice-bath for an additional hour, 25 ml. of water are added and then a solution of 5 ml. of concentrated hydrochloric acid in 25 ml. of water (CO₂ evolution). The strongly acid solution is saturated with sodium chloride and extracted with 50 ml. of ethyl acetate (four times). The organic phases are combined, dried (MgSO₄), filtered and the solvent evaporated to give 6.0 g. (90%) of colorless syrupy 110 1 - (3 - acetylthio - 1 - oxopropyl) - trans - 4 - methoxy - L - proline. This acid is dissolved in 25 ml. of ethyl acetate and treated with 4.7 g. of dicyclohexylamine to give a solution which rapidly becomes a solid mass. An additional 15 ml. of ethyl acetate are added 115 and the mixture is digested on a steam bath, cooled and filtered to give 8.7 g. of the dicyclohexylamine salt, m.p. 170-172°. After crystallization from 60 ml. of acetonitrile the colorless solid weighs 8.3 g. (75%) m.p. 171-173°; $[\alpha]^{25}_D -35^\circ$ (c, 1% in EtOH).
120 The dicyclohexylamine salt is converted to 1 - (3 - acetylthio - 1 - oxopropyl) - trans - 4 - methoxy - L - proline by suspending 8.0 g. in 60 ml. of ethyl acetate cooled in an ice bath and treating portionwise with 60 ml. of 10% potassium bisulfate. The clear layers 125 are separated and the aqueous portion extracted 130

with 60 ml. of ethyl acetate (2X). The organic phases are combined, dried ($MgSO_4$), filtered and the solvent is evaporated to give 4.6 g. (80%) of a colorless syrup.

5 **Example 2**

1 - (3 - Mercapto - 1 - oxopropyl) - trans - 4 - methoxy - L - proline

To the 1 - (3 - acetylthio - 1 - oxopropyl) - trans - 4 - methoxy - L - proline obtained in Example 1 (4.6 g., 0.017 mole) is added a cold solution of 9 ml. of concentrated ammonia in 22 ml. of water. The base dissolves in about 30 minutes and the resulting solution (under Argon) is allowed to stand for 2 hours at room temperature. This solution is cooled, extracted with 25 ml. of ethyl acetate (2X) and the ethyl acetate extract is discarded. The solution is again layered with 25 ml of ethyl acetate and acidified with 17 ml. of 1:1 hydrochloric acid. The mixture is shaken, separated and the aqueous phase extracted with 25 ml. of ethyl acetate (3X). The organic phases are combined, dried ($MgSO_4$), filtered and the solvent removed on the rotary evaporator to give 2.3 g. (59%) of colorless syrup, 1 - (3 - mercapto - 1 - oxopropyl) - trans - 4 - methoxy - L - proline, $[\alpha]^{25}_D - 60^\circ$ (c, 1% in EtOH); R, 0.49 (MeOH on silica gel, visualized with nitroprusside reagent).

Anal. Calc'd. for $C_9H_{15}NO_4S$. 1/4H₂O: C, 45.46; H, 6.57; N, 5.87; S, 13.48.

Found: C, 45.42; H, 6.78; N, 5.96; S, 13.27.

30 An additional 1.1 g. of product (total 3.4 g., 87%) is obtained by saturating the aqueous phase with sodium chloride and extracting with 25 ml. of ethyl acetate (2X).

The sodium salt is formed by treating the syrup 35 with aqueous sodium bicarbonate and freeze drying.

Example 3

(trans) - 1 - [D - 3 - (Acetylthio) - 2 - methyl - 1 - oxopropyl] - 4 - methoxy - L - proline

A solution of 4.3 g. (0.029 mole) of trans - 4 - 40 methoxy - L - proline in 50 ml. of water is stirred, cooled to 5° and 3 g. of sodium carbonate are added. This solution is treated with 5.2 g. (0.029 mole) of D - 3 - (acetylthio) - 2 - methylpropionyl chloride in 5 ml. of ether during the course of 10 minutes with the 45 intermittent addition of 3 g. of sodium carbonate to maintain the pH at about 8.0. This mixture is stirred in the ice-bath for 1.5 hours, 25 ml. of water are added and then a solution of 6 ml. of concentrated hydrochloric acid in 25 ml. of water (CO_2 evolution).

50 The resulting strongly acidic solution is extracted with 50 ml. of ethyl acetate (four times). The organic phases are combined, dried ($MgSO_4$), filtered and the solvent evaporated to give 6.1 g. (73%) of (trans) - 1 - [D - 3 - (acetylthio) - 2 - methyl - 1 - oxopropyl] - 4 - 55 methoxy - L - proline as a pale yellow syrupy residue.

This acid is dissolved in 50 ml. of ethyl acetate and treated with a solution of 4.0 g. of dicyclohexylamine in 20 ml. of ethyl acetate. The product begins to crystallize from the solution in about a minute. After 60 cooling overnight the nearly colorless solid is filtered and dried, yield 6.7 g., m.p. 175-177°; $[\alpha]^{25}_D - 55^\circ$ (c, 1% in EtOH). Following crystallization from 60 ml. of acetonitrile the nearly colorless solid dicyclohexylamine salt weighs 5.6 g. (41%), m.p. 179-181°, $[\alpha]^{25}_D - 62^\circ$ (c, 1% in EtOH).

The dicyclohexylamine salt is converted to the acid by suspending 5.5 g. in 50 ml. of ethyl acetate, cooling in an ice-bath and treating with 50 ml. of 10% potassium bisulfate. The layers are separated and

70 the aqueous portion is extracted with 50 ml. of ethyl acetate (2X). The organic phases are combined, dried ($MgSO_4$), filtered and the solvent evaporated to give 3.4 g. (41%) of nearly colorless (trans) - 1 - [D - 3 - (acetylthio) - 2 - methyl - 1 - oxopropyl] - 4 - methoxy - 75 - L - proline as a syrup.

Example 4

(trans) - 4 - Methoxy - 1 - (D - 3 - mercapto - 2 - methyl - 1 - oxopropyl) - L - proline

To (trans) - 1 - [D - 3 - (acetylthio) - 2 - methyl - 1 - oxopropyl] - 4 - methoxy - L - proline (3.4 g.) is added a cold solution of 8 ml. of concentrated ammonia in 20 ml. of water. The base dissolves in about 10 minutes and the resulting solution (under Argon) is allowed to stand at room temperature for two hours.

85 This solution is cooled, extracted with 20 ml. of ethyl acetate (2X), layered with 20 ml. of ethyl acetate and acidified with 15 ml of 1:1 hydrochloric acid. This mixture is saturated with sodium chloride, the layers are separated and the aqueous phase is extracted

90 with 20 ml. of ethyl acetate (3X). The organic phases are combined, dried ($MgSO_4$), filtered and the solvent evaporated to give 2.9 g. (100%) of nearly colorless (trans) - 4 - methoxy - 1 - (D - 3 - mercapto - 2 - methyl - 1 - oxopropyl) - L - proline, $[\alpha]^{25}_D - 80^\circ$ (c, 1%

95 in EtOH); R, 0.53 (MeOH on silica gel, visualized with nitroprusside reagent).

Anal. calc'd. for $C_{10}H_{17}NO_4S$. 1/4H₂O: C, 47.69; H, 6.83; N, 5.56; S, 12.73.

Found: C, 47.90; H, 6.84; N, 5.85; S, 12.76.

100 **Example 5**

(trans) - 1 - [D - 3 - (Acetylthio) - 2 - methyl - 1 - oxopropyl] - 4 - ethoxy - L - proline

Utilizing the procedure described in Example 3 but substituting an equivalent quantity of trans - 4 - 105 ethoxy - L - proline (J. Med. Chem., 10, 1161 (1967)), for (trans) - 4 - methoxy - L - proline, (trans) - 1 - [D - 3 - (acetylthio) - 2 - methyl - 1 - oxopropyl] - 4 - ethoxy - L - proline is obtained. The product is purified as the dicyclohexylamine salt, m.p. 170-172° (crystallized 110 from isopropyl alcohol); $[\alpha]^{25}_D - 64^\circ$ (c, 1% in EtOH).

This salt (7.75 g.) is converted to the free acid by treating with potassium bisulfate solution as described in Example 4 to give 4.85 g. of nearly colorless (trans) - 1 - [D - 3 - (acetylthio) - 2 - methyl - 1 - 115 oxopropyl] - 4 - ethoxy - L - proline as a syrup.

Example 6

(trans) - 4 - Ethoxy - 1 - (D - 3 - mercapto - 2 - methyl - 1 - oxopropyl) - L - proline

To the material from Example 5(4.85 g.) is added a 120 cold solution of 9 ml. of concentrated ammonia in 22 ml. of water (under Argon). The mixture is treated in the same manner as Example 4 to give 4.2 g. (100%) of nearly colorless (trans) - 4 - ethoxy - 1 - (D - 3 - mercapto - 2 - methyl - 1 - oxopropyl) - L - proline as a 125 syrup, $[\alpha]^{25}_D - 80^\circ$ (c, 1% in EtOH); R, 0.64 (MeOH on silica gel, visualized with nitroprusside reagent).

Anal. calc'd. for $C_{11}H_{19}NO_4S$: C, 50.55; H, 7.33; N, 5.36; S, 12.27.

Found: C, 50.34; H, 7.34; N, 5.39; S, 12.11.

130 **Example 7**

(*cis*) - 1 - [*D* - 3 - (*Acetylthio*) - 2 - methyl - 1 - oxopropyl] - 4 - methoxy - L - proline
a) N - Carbobenzoyloxy - *cis* - 4 - hydroxy - L - proline
N - Carbobenzoyloxy - 4 - keto - L - proline (10 g., 0.038) is dissolved in 300 ml. of methanol and reduced with 5.8 g. (0.15 mole) of sodium borohydride in 20 ml. of water as described in JACS, 79, 189 (1957) to give 8.7 g. of a foamy product. This material is dissolved in 30 ml. of ethanol, treated with 3.5 g. of cyclohexylamine in some ethanol, and diluted to 500 ml. with ether. On seeding and rubbing, the crystalline cyclohexylamine salt separates rapidly to give 10.8 g.; m.p. 163-165°. This cyclohexylamine salt is then treated with 30 ml. of 2N HCl and extracted with ethyl acetate (4 x 50 ml.) to yield as a glass-like material 8 g. of *N* - carbobenzoyloxy - *cis* - 4 - hydroxy - L - proline.
b) N - Carbobenzoyloxy - *cis* - 4 - methoxy - L - proline, methyl ester
20 *N* - Carbobenzoyloxy - *cis* - 4 - hydroxy - L - proline (13.9 g., 0.052 mole) is treated with 40 g. of silver oxide and 40 ml. of methyl iodide (2x) in acetone (100 ml. initially, then 120 ml.) as described in Example 1(b) to yield 17.5 g. (100%) of *N* - carbobenzoyloxy - *cis* - 4 - methoxy - L - proline, methyl ester.
c) N - Carbobenzoyloxy - *cis* - 4 - methoxy - L - proline
The *N*-carbobenzoyloxy - *cis* - 4 - methoxy - L - proline, methyl ester (17.5 g., approximately 0.052 mole) is dissolved in 135 ml. of methanol, treated dropwise at -1° to 4° with 32 ml. (0.064 mole) of 2N sodium hydroxide, then kept at 0° for one hour, and at room temperature overnight. After removing about half of the solvent on a rotary evaporator, the solution is diluted with 300 ml of water, washed with ether (wash discarded), acidified while cooling with 12.5 ml. of 1:1 hydrochloric acid to pH 2, and extracted with ethyl acetate (4 x 150 ml.). The extracts are combined, dried (MgSO_4), filtered and the solvent evaporated to give 15 g. of an orange-yellow syrup. The latter is dissolved in 60 ml. of ethanol, treated with 6 g. of cyclohexylamine in 10 ml. of ethanol and diluted to 900 ml. with ether. On seeding and rubbing, crystalline *N* - carbobenzoyloxy - *cis* - 4 - methoxy - L - proline, cyclohexylamine salt separates: weight after cooling overnight 10.2 g., m.p. 148-150° (s. 144°), $[\alpha]^{25}_{D} -35^{\circ}$ (c, 1% in ethanol). Following recrystallization from 40 ml. of acetonitrile, the nearly colorless solid weighs 8.8 g., m.p. 150-152° (s. 145°), $[\alpha]^{25}_{D} -34^{\circ}$ (c, 1% in ethanol).
The cyclohexylamine salt is treated with hydrochloric acid to yield 6.9 g. (48%) of *N* - carbobenzoyloxy - *cis* - 4 - methoxy - L - proline as a pale yellow viscous syrup, $[\alpha]^{25}_{D} -32^{\circ}$ (c, 1% in ethanol).
d) cis - 4 - Methoxy - L - proline
A mixture of 6.8 g. of *N* - carbobenzoyloxy - *cis* - 4 - methoxy - L - proline, 210 ml. of 2:1 methanol and water, and 2.3 g. of 5% Pd - C is placed on a hydrogenator at 3 atmospheres of hydrogen for four hours. The mixture is filtered to remove the catalyst and the filtrate evaporated to give 3.15 g. of a grayish solid; m.p. 218-220° (dec.). A sample is crystallized from methanol-ether to yield colorless *cis* - 4 - methoxy - L - proline, m.p. 224-226° (dec.), $[\alpha]^{25}_{D} -42^{\circ}$ (c, 1% in methanol).

Anal. Calc'd. for $\text{C}_6\text{H}_{11}\text{NO}_3$: C, 49.64; H, 7.74; N, 9.65
Found: C, 49.63; H, 7.71; N, 9.54.
e) (cis) - 1 - [*D* - 3 - (*Acetylthio*) - 2 - methyl - 1 - oxopropyl] - 4 - methoxy - L - proline
70 *cis* - 4 - Methoxy - L - proline (3 g., 0.021 mole) and 4.2 g. (0.023 mole) of *D* - 3 - acetylthio - 2 - methylpropionyl chloride in 5 ml. of ether are reacted in 60 ml. of water in the presence of sodium bicarbonate as described in Example 3. Approximately 20 ml. of 25% sodium carbonate (w/v) is required to bring the pH initially to 8.5 and to maintain it at 7.5 to 8.4 during the acylation. The resulting crude viscous product (6.4 g.) is dissolved in 50 ml. of ethyl acetate and treated with 3.9 g. of dicyclohexylamine in 20 ml. of ethyl acetate. The product crystallizes from the solution and is filtered and dried to yield 6.6 g. of dicyclohexylamine salt, m.p. 172-174° (s. 170°), $[\alpha]^{25}_{D} -60^{\circ}$ (c, 1% in ethanol). 6.5 g. of this material is recrystallized from 35 ml. of acetonitrile to yield 6 g. of colorless solid dicyclohexylamine salt; m.p. 173-175° (s. 170°), $[\alpha]^{25}_{D} -60^{\circ}$ (c, 1% in ethanol).
Anal. Calc'd. for $\text{C}_{12}\text{H}_{19}\text{NO}_5\text{S} \cdot \text{C}_{12}\text{H}_{23}\text{N}$: C, 61.24; H, 9.00; N, 5.95; S, 6.81
Found: C, 61.16; H, 8.81; N, 5.95; S, 6.67.
90 Utilizing the procedure of Example 3, the dicyclohexylamine salt is converted to the acid by suspending 5.9 g. in 60 ml. of ethyl acetate, cooling in an ice-bath and treating with 60 ml. of 10% potassium bisulfate. The layers are separated and the aqueous phase is extracted with 50 ml. of ethyl acetate (4x). The organic phases are combined, dried (MgSO_4), filtered and the solvent evaporated to give 3.5 g. (60%) of colorless (*cis*) - 1 - [*D* - 3 - (*acetylthio*) - 2 - methyl - 1 - oxopropyl] - 4 - methoxy - L - proline;
95 100 m.p. 90-92° (triturated with ether), $[\alpha]^{25}_{D} -139^{\circ}$ (c, 1% in ethanol), R_f 0.63 (methanol on silica gel).
Anal. Calc'd. for $\text{C}_{12}\text{H}_{19}\text{NO}_5\text{S}$: C, 49.81; H, 6.62; N, 4.84
105 *Example 8*
(*cis*) - 4 - Methoxy - 1 - (*D* - 3 - mercapto - 2 - methyl - 1 - oxopropyl) - L - proline
The (*cis*) - 1 - [*D* - 3 - (*acetylthio*) - 2 - methyl - 1 - oxopropyl] - 4 - methoxy - L - proline (2.9 g., 0.011 mole) is hydrolyzed in 15 ml. water containing 6.5 ml. of concentrated ammonia as described in Example 4 to give 2.3 g. (93%) of extremely viscous (*cis*) - 4 - methoxy - 1 - (*D* - 3 - mercapto - 2 - methyl - 1 - oxopropyl) - L - proline which becomes waxy on standing; $[\alpha]^{25}_{D} -88^{\circ}$ (c, 1% in ethanol).
Example 8a
(*cis*) - 4 - Methoxy - 1 - (*D* - 3 - mercapto - 2 - methyl - 1 - oxopropyl) - L - proline, 1 - adamantanamine salt
A solution of 0.55 g. (0.0022 mole) of *cis* - 4 - methoxy - 1 - (*D* - 3 - mercapto - 2 - methyl - 1 - oxopropyl) - L - proline in 15 ml. of ethyl acetate is treated under an atmosphere of argon with a warm solution of 0.34 g. (0.0022 mole) of 1 - adamantanamine in 10 ml. of ethyl acetate to precipitate the salt. After three hours in the cold, the colorless solid is filtered under argon (solvent held tenaciously), washed with some cold ethyl acetate, and dried in vacuo for twenty hours to yield 0.7 g. (79%) of (*cis*) - 4 - methoxy - 1 - (*D* - 3 - mercapto - 2 - methyl - 1 - oxopropyl) - L - proline, 1 - adamantanamine salt,
120 125
130

m.p. 215-217° (s. 210°, dec. 220°), $[\alpha]^{25}_D - 60^\circ$ (c, 1% in methanol).

Example 9

(*cis*) - 4 - *Methoxy* - 1 - [*3* - *mercapto* - 2 - *methyl* - 1 - 5 *oxopropyl*] - *L* - *proline*

a) (*cis*) - 4 - *Methoxy* - 1 - [*3* - (*benzoylthio*) - 2 - *methyl* - 1 - *oxopropyl*] - *L* - *proline*

Interaction of 3 - benzoylthio - 2 - methylpropionyl acid chloride (prepared by treating 3 - benzoylthio - 2 - methylpropanoic acid with thionyl chloride) with *cis* - 4 - methoxy - *L* - proline according to the general procedure of Example 3 yields (*cis*) - 4 - *methoxy* - 1 - [*3* - (*benzoylthio*) - 2 - *methyl* - 1 - *oxopropyl*] - *L* - *proline*.

b) (*cis*) - 4 - *Methoxy* - 1 - [*3* - *mercapto* - 2 - *methyl* - 1 - *oxopropyl*] - *L* - *proline*

Hydrolysis of (*cis*) - 2 - *methoxy* - 1 - [*3* - (*benzoylthio*) - 2 - *methyl* - 1 - *oxopropyl*] - 2 - *proline* with an aqueous ammonia solution according to the general procedure of Example 4 yields (*cis*) - 4 - *methoxy* - 1 - (*3* - *mercapto* - 2 - *methyl* - 1 - *oxopropyl*) - *L* - *proline*.

Example 10

(*trans*) - 1 - [*D* - 3 - (*Acetylthio*) - 2 - *methyl* - 1 - *oxopropyl*] - 4 - *propoxy* - *L* - *proline*

a) *N* - *acetyl* - *trans* - 4 - *propoxy* - *L* - *proline*, *propyl ester*

Interaction of 30 g. of *N* - *acetyl* - *trans* - 4 - *hydroxy* - *L* - *proline* from Example 1(a) with 110 g. of silver oxide and 110 ml. of propyl iodide in 300 ml. of acetone according to the procedure of Example 1(b) gives 19.6 g. (41%) of pale yellow-orange *N* - *acetyl* - *trans* - 4 - *propoxy* - *L* - *proline*, *propyl ester*; b.p. 155-165° (0.2 mm.).

b) *N* - *Acetyl* - *trans* - 4 - *propoxy* - *L* - *proline*

A solution of 6 g. (0.15 mole) of sodium hydroxide in 150 ml. of water is added to 19.4 g. (0.075 mole) of *N* - *acetyl* - *trans* - 4 - *propoxy* - *L* - *proline*, *propyl ester* to give a pale orange solution. After standing overnight at room temperature, the solution is extracted with 60 ml. of ethyl acetate (wash discarded), then acidified with 1:1 hydrochloric acid, then saturated with sodium chloride and extracted with 50 ml. of chloroform (3x). The organic phases are combined, dried (MgSO_4), filtered and the solvent evaporated to give 12.6 g. of a brown oil. The latter is dissolved in 80 ml. of ethyl acetate and treated with a solution of 10.7 g. of dicyclohexylamine in 20 ml. of ethyl acetate. The salt crystallizes at room temperature. After standing overnight under refrigeration, the dicyclohexylamine salt is filtered and washed with cold ethyl acetate to give 17.3 g. of nearly colorless solid dicyclohexylamine salt; m.p. 148-153°. Recrystallization of this material from 125 ml. of ethyl acetate gives 14.5 g. of colorless dicyclohexylamine salt; m.p. 157-159°, $[\alpha]^{25}_D - 30^\circ$ (c, 1% in ethanol).

Anal. Calc'd. for $\text{C}_{10}\text{H}_{17}\text{NO}_4 \cdot \text{C}_{12}\text{H}_{23}\text{N}$: C, 66.63; H, 10.17; N, 7.07.

Found: C, 66.47; H, 10.22; N, 7.06.

The dicyclohexylamine salt (14.4 g.) is converted to the free acid by pulverizing, suspending in 100 ml. of ethyl acetate and treating the slurry portionwise with 100 ml. of 10% potassium bisulfate. The organic phase is separated and the aqueous phase is extracted with 100 ml. of ethyl acetate (2x). The

organic phases are combined, dried (MgSO_4), filtered, and the solvent evaporated to give 6.7 g. (41%) of *N* - *acetyl* - *trans* - 4 - *propoxy* - *L* - *proline* as a pale brown liquid.

c) (*trans*) - 1 - [*D* - 3 - (*Acetylthio*) - 2 - *methyl* - 1 - *oxopropyl*] - 4 - *propoxy* - *L* - *proline*

N - *Acetyl* - *trans* - 4 - *propoxy* - *L* - *proline* (6.4 g., 0.03 mole) is treated with a solution of 10 g. of concentrated sulfuric acid in 100 ml. of water and the

resulting solution is stirred and refluxed for three hours. The solution is then cooled to 15°, treated portionwise with 12 g. of sodium carbonate to bring the pH to 8.0, and then treated with a solution of 5.4 g. (0.03 mole) of *D* - 3 - *acetylthio* - 2 - *methylpropionyl*

chloride in 5 ml. of ether during 10 minutes while 7 g. of sodium carbonate are added to maintain the pH at about 8.0. The mixture is then stirred in the ice-bath for 30 minutes and at room temperature for one hour. The product is then isolated and purified as a dicyclohexylamine salt according to the procedure of Example 3 to yield 6.3 g. of dicyclohexylamine salt; m.p. 165-167° (from acetonitrile), $[\alpha]^{25}_D - 56^\circ$ (c, 1% in ethanol).

Anal. Calc'd. for $\text{C}_{14}\text{H}_{23}\text{NO}_5\text{S} \cdot \text{C}_{12}\text{H}_{23}\text{N}$: C, 62.62;

90 H, 9.30; N, 5.61; S, 6.43.

Found: C, 62.35; H, 9.48; N, 5.88; S, 6.45.

The dicyclohexylamine salt (6.1 g.) is converted to the free acid by suspending in 60 ml. of ethyl acetate, cooling in an ice-bath and treating with 60 ml. of 10% potassium bisulfate. The layers are separated and the aqueous phase is extracted with 60 ml. of ethyl acetate (2x). The organic phases are combined, dried (MgSO_4), filtered and the solvent evaporated to give 4.0 g. (42%) of (*trans*) - 1 - [*D* - 3 - (*acetylthio*) - 2 - 100 *methyl* - 1 - *oxopropyl*] - 4 - *propoxy* - *L* - *proline* as a nearly colorless syrup.

Example 11

1 - (*D* - 3 - *Mercapto* - 2 - *methyl* - 1 - *oxopropyl*) - *trans* - 4 - *propoxy* - *L* - *proline*

Hydrolysis of 4.0 g. of (*trans*) - 1 - [*D* - 3 - (*acetylthio*) - 2 - *methyl* - 1 - *oxopropyl*] - 4 - *propoxy* - *L* - *proline* with 8 ml. of concentrated ammonia in 20 ml. of water under argon according to the procedure of Example 4 gives 3.42 g. (97%) of 1 - (*D* - 3 - *mercapto* - 110 2 - *methyl* - 1 - *oxopropyl*) - *trans* - 4 - *propoxy* - *L* - *proline* as a nearly colorless syrup, $[\alpha]^{25}_D - 72^\circ$ (c, 1% in ethanol).

Anal. Calc'd. for $\text{C}_{12}\text{H}_{22}\text{NO}_4\text{S} \frac{1}{4}\text{H}_2\text{O}$: C, 51.49; H, 7.74; N, 5.05; S, 11.46

115 Found: C, 51.66; H, 7.76; N, 5.94; S, 10.51.

Example 12

1 - (*3* - *Mercapto* - 1 - *oxopropyl*) - *cis* - 4 - *methoxy* - *L* - *proline*

a) 1 - (*3* - *Acetylthio* - 1 - *oxopropyl*) - *cis* - 4 - *methoxy*

120 - *L* - *proline*

Following the procedure of Example 1(d), *cis* - 4 - *methoxy* - *L* - *proline* is treated with a solution of 3 - *acetylthiopropyl* chloride in the presence of sodium carbonate to yield 1 - (*3* - *acetylthio* - 1 - *oxopropyl*) - 125 *cis* - 4 - *methoxy* - *L* - *proline*.

b) 1 - (*3* - *Mercapto* - 1 - *oxopropyl*) - *cis* - 4 - *methoxy* - *L* - *proline*

The 1 - (*3* - *acetylthio* - 1 - *oxopropyl*) - *cis* - 4 - *methoxy* - *L* - *proline* is hydrolyzed with an aqueous 130 solution of ammonia according to the procedure of

Example 2 to yield 1 - (3 - mercapto - 1 - oxopropyl) - cis - 4 - methoxy - L - proline.

Example 13

(*cis*) - 4 - Ethoxy - 1 - (*D* - 3 - mercapto - 2 - methyl - 1 - 5 oxopropyl) - L - proline

a) *cis* - 4 - Ethoxy - L - proline

Following the procedure of Example 7, parts (a) to (d) but substituting ethyl iodide for the methyl iodide in part (b), one obtains (*cis*) - 4 - ethoxy - L - proline.

10 b) (*cis*) - 1 - [*D* - 3 - (*Acetylthio*) - 2 - methyl - 1 - oxopropyl] - 4 - ethoxy - L - proline

The (*cis*) - 4 - ethoxy - L - proline is reacted with a solution of *D* - 3 - acetylthio - 2 - methylpropionyl chloride in the presence of sodium carbonate

15 according to the procedure of Example 7(e) to yield (*cis*) - 1 - [*D* - 3 - (*Acetylthio*) - 2 - methyl - 1 - oxopropyl] - 4 - ethoxy - L - proline.

c) (*cis*) - 4 - Ethoxy - 1 - (*D* - 3 - mercapto - 2 - methyl - 1 - oxopropyl) - L - proline

20 The *cis* - 1 - [*D* - 3 - (*Acetylthio*) - 2 - methyl - 1 - oxopropyl] - 4 - ethoxy - L - proline is hydrolyzed with a solution of aqueous ammonia according to the procedure of Example 8 to yield (*cis*) - 4 - ethoxy - 1 - (*D* - 3 - mercapto - 2 - methyl - 1 - oxopropyl) - L -

25 proline.

Example 14

(*trans*) - 4 - Allyloxy - 1 - (*D* - 3 - mercapto - 2 - methyl - 1 - oxopropyl) - L - proline

a) (*trans*) - 4 - Allyloxy - L - proline

30 Using the procedure of Example 1, part b, but substituting allyl bromide for methyl iodide gives the (*trans*) - N - acetyl - 4 - allyl - L - proline, allyl ester. The latter is then hydrolyzed as described in Example 1, part c, to give (*trans*) - 4 - allyloxy - L - proline.

35 b) (*trans*) - 1 - [*D* - 3 - (*Acetylthio*) - 2 - methyl - 1 - oxopropyl] - 4 - allyloxy - L - proline

Interaction of the (*trans*) - 4 - allyloxy - L - proline from part (a) with an equivalent quantity of *D* - 3 - (*Acetylthio*) - 2 - methylpropionyl chloride according

40 to the procedure of Example 3 gives (*trans*) - 1 - [*D* - 3 - (*Acetylthio*) - 2 - methyl - 1 - oxopropyl] - 4 - allyloxy - L - proline.

c) (*trans*) - 4 - Allyloxy - 1 - (*D* - 3 - mercapto - 2 - methyl - 1 - oxopropyl) - L - proline

45 The (*trans*) - 1 - [*D* - 3 - (*Acetylthio*) - 2 - methyl - 1 - oxopropyl] - 4 - allyloxy - L - proline is hydrolyzed with an aqueous ammonia solution according to the procedure of Example 4 to give (*trans*) - 4 - allyloxy - 1 - (*D* - 3 - mercapto - 2 - methyl - 1 - oxopropyl) - L -

50 proline.

Example 15

1 - (*D* - 3 - Mercapto - 2 - methyl - 1 - oxopropyl) - trans - 4 - propargyloxy - L - proline

a) (*trans*) - 4 - propargyloxy - L - proline

55 Using the procedure of Example 1, part b, but substituting propargyl bromide for methyl iodide gives the (*trans*) - N - acetyl - 4 - propargyl - L - proline, propargyl ester. The latter is then hydrolyzed as described in Example 1, part c, to give the (*trans*) - 4 -

60 propargyloxy - L - proline.

b) (*trans*) - 1 - [*D* - 3 - (*Acetylthio*) - 2 - methyl - 1 - oxopropyl] - 4 - propargyloxy - L - proline

Interaction of the (*trans*) - 4 - propargyloxy - L - proline from part (a) with an equivalent quantity of *D*

65 - 3 - (*Acetylthio*) - 2 - methylpropionyl chloride

according to the procedure of Example 3 gives (*trans*) - 1 - [*D* - 3 - (*Acetylthio*) - 2 - methyl - 1 - oxopropyl] - 4 - propargyloxy - L - proline.

c) 1 - (*D* - 3 - Mercapto - 2 - methyl - 1 - oxopropyl) - trans - 4 - propargyloxy - L - proline

The (*trans*) - 1 - [*D* - 3 - (*Acetylthio*) - 2 - methyl - 1 - oxopropyl] - 4 - propargyloxy - L - proline is hydrolyzed with an aqueous ammonia solution according to the procedure of Example 4 to give 1 - [*(D* - 3 - mercapto - 2 - methyl - 1 - oxopropyl)] - trans - 4 - propargyloxy - L - proline.

Example 16

(*trans*) - 4 - Benzyloxy - 1 - (*D* - 3 - mercapto - 2 - methyl - 1 - oxopropyl) - L - proline

a) (*trans*) - 4 - Benzyloxy - L - proline

Using the procedure of Example 1, part b, but substituting benzyl chloride for methyl iodide gives the (*trans*) - N - acetyl - 4 - benzyloxy - L - proline, benzyl ester. The latter is then hydrolyzed as described in Example 1, part c, to give the (*trans*) - 4 - benzyloxy - L - proline.

b) (*trans*) - 1 - [*D* - 3 - (*Acetylthio*) - 2 - methyl - 1 - oxopropyl] - 4 - benzyloxy - L - proline

Interaction of the (*trans*) - 4 - benzyloxy - L - proline from part (a) with an equivalent quantity of *D* - 3 - (*Acetylthio*) - 2 - methylpropionyl chloride according to the procedure of Example 3 gives (*trans*) - 1 - [*D* - 3 - (*Acetylthio*) - 2 - methyl - 1 - oxopropyl] - 4 - benzyloxy - L - proline.

c) (*trans*) - 4 - Benzyloxy - 1 - (*D* - 3 - mercapto - 2 - methyl - 1 - oxopropyl) - L - proline

The (*trans*) - 1 - [*D* - 3 - (*Acetylthio*) - 2 - methyl - 1 - oxopropyl] - 4 - benzyloxy - L - proline is hydrolyzed with an aqueous ammonia solution according to the procedure of Example 4 to give (*trans*) - 4 - benzyloxy - 1 - (*D* - 3 - mercapto - 2 - methyl - 1 - oxopropyl) - L -

50 proline.

Example 17

1 - (*D* - 3 - Mercapto - 2 - methyl - 1 - oxopropyl) - trans - 4 - phenethyloxy - L - proline

a) (*trans*) - 4 - Phenethyloxy - L - proline

Using the method of Example 1, part b, but substituting phenethyl bromide for methyl iodide gives the (*trans*) - N - acetyl - 4 - phenethyloxy - L - proline,

110 phenethyl ester. The latter is then hydrolyzed as described in Example 1, part c, to give the (*trans*) - 4 - phenethyloxy - L - proline.

b) (*trans*) - 1 - [*D* - 3 - (*Acetylthio*) - 2 - methyl - 1 - oxopropyl] - 4 - phenethyloxy - L - proline

115 Interaction of the (*trans*) - 4 - phenethyloxy - L - proline from part (a) with an equivalent quantity of *D* - 3 - (*Acetylthio*) - 2 - methylpropionyl chloride according to the procedure of Example 3 gives (*trans*) - 1 - [*D* - 3 - (*Acetylthio*) - 2 - methyl - 1 -

120 oxopropyl] - 4 - phenethyloxy - L - proline.

c) 1 - (*D* - 3 - Mercapto - 2 - methyl - 1 - oxopropyl) - trans - 4 - phenethyloxy - L - proline

The (*trans*) - 1 - [*D* - 3 - (*Acetylthio*) - 2 - methyl - 1 - oxopropyl] - 4 - phenethyloxy - L - proline is hydrolyzed with an aqueous ammonia solution according to the procedure of Example 4 to give 1 - (*D* - 3 - mercapto - 2 - methyl - 1 - oxopropyl) - trans - 4 - phenethyloxy - L - proline.

Example 18

130 1 - (*D* - 3 - Mercapto - 2 - methyl - 1 - oxopropyl) - cis -

3-methoxy-L-proline

a) 1-[D-3-(Acetylthio)-2-methyl-1-oxopropyl]-cis-3-methoxy-L-proline

Utilizing the procedure described in Example 7,

5 but substituting an equivalent quantity of (cis)-3-methoxy-L-proline (J. Amer. Chem. Soc. 85, 3863 (1963)) for the (cis)-4-methoxy-L-proline, 1-[D-3-(acetylthio)-2-methyl-1-oxopropyl]-cis-3-methoxy-L-proline is obtained.

10 b) 1-(D-3-Mercapto-2-methyl-1-oxopropyl)-cis-3-methoxy-L-proline

The 1-[D-3-(acetylthio)-2-methyl-1-oxopropyl]-cis-3-methoxy-L-proline is hydrolyzed with an aqueous ammonia solution according to the 15 procedure of Example 4 to yield 1-(D-3-mercaptoproline).

Example 19

1-(D,L-3-Mercapto-2-methyl-1-oxopropyl)-

20 trans-3-methoxy-L-proline

a) 1-[D,L-3-(Acetylthio)-2-methyl-1-oxopropyl]-trans-3-methoxy-L-proline

Interaction of equivalent quantities of (trans)-3-methoxy-L-proline (J. Amer. Chem. Soc. 85, 3863 25 (1963)) and D,L-3-(acetylthio)-2-methylpropionyl chloride according to the procedure described in Example 3 gives 1-[D,L-3-(acetylthio)-2-methyl-1-oxopropyl]-trans-3-methoxy-L-proline.

b) 1-(D,L-3-Mercapto-2-methyl-1-oxopropyl)-

30 trans-3-methoxy-L-proline

The 1-[D,L-3-(acetylthio)-2-methyl-1-oxopropyl]-trans-3-methoxy-L-proline is hydrolysed with an aqueous ammonia solution according to the procedure of Example 4 to yield 1-(D,L-3-35 mercapto-2-methyl-1-oxopropyl)-trans-3-methoxy-L-proline.

Example 20

1-(D-3-Mercapto-2-methyl-1-oxopropyl)-cis-4-methylothio-L-proline

40 a) 1-[D-3-(Acetylthio)-2-methyl-1-oxopropyl]-cis-4-methylothio-L-proline

Interaction of equivalent quantities of cis-4-methylothio-L-proline [J. Amer. Chem. Soc., 79, 185 (1957)] and D-3-(acetylthio)-2-methylpropionyl

45 chloride according to the procedure of Example 3 gives 1-[D-3-(acetylthio)-2-methyl-1-oxopropyl]-cis-4-methylothio-L-proline.

b) 1-(D-3-Mercapto-2-methyl-1-oxopropyl)-cis-4-methylothio-L-proline

50 The 1-[D-3-(acetylthio)-2-methyl-1-oxopropyl]-cis-4-methylothio-L-proline is hydrolysed with an aqueous ammonium solution according to the procedure of Example 4 to give 1-(D-3-mercaptoproline).

55 -L-proline.

Example 21

1-(D-3-Mercapto-2-methyl-1-oxopropyl)-trans-4-methylothio-L-proline

60 a) 1-[D-3-(Acetylthio)-2-methyl-1-oxopropyl]-trans-4-methylothio-L-proline

Interaction of equivalent quantities of trans-4-methylothio-L-proline [J. Amer. Chem. Soc., 79, 185 (1957)] and D-3-(acetylthio)-2-methylpropionyl chloride according to the procedure of Example 3

65 gives 1-[D-3-(acetylthio)-2-methyl-1-oxopropyl]-trans-4-methylothio-L-proline.

ropyl]-trans-4-methylothio-L-proline.

b) 1-(D-3-Mercapto-2-methyl-1-oxopropyl)-trans-4-methylothio-L-proline

The 1-[D-3-(acetylthio)-2-methyl-1-oxopropyl]-trans-4-methylothio-L-proline is hydrolyzed with an aqueous ammonia solution according to the procedure of Example 4 to give 1-(D-3-mercaptoproline).

Example 22

1-(D-3-Mercapto-3-methyl-1-oxopropyl)-cis-4-(4-pentenylthio)-L-proline

a) (cis)-4-(4-Pentenylthio)-L-proline

Interaction of (cis)-N-acetyl-4-mercaptoproline [Chem. Pharm. Bull., 20, 543 (1972)] with 5-bromo-1-pentene in acetone in the presence of silver oxide according to the procedure of Example 1, part b, gives the (cis)-N-acetyl-4-(4-pentenylthio)-L-proline, 4-pentenyl ester. The latter is then hydrolyzed as described in Example 1, part c, to give the cis-4-(4-pentenylthio)-L-proline.

b) 1-[D-3-(Acetylthio)-3-methyl-1-oxopropyl]-cis-4-(4-pentenylthio)-L-proline

Interaction of cis-4-(4-pentenylthio)-L-proline 90 with an equivalent amount of D-3-(acetylthio)-3-methyl-propionyl chloride according to the procedure of Example 3 gives 1-[D-3-(acetylthio)-3-methyl-1-oxopropyl]-cis-4-(4-pentenylthio)-L-proline.

95 c) 1-(D-3-Mercapto-3-methyl-1-oxopropyl)-cis-4-(4-pentenylthio)-L-proline

The 1-[D-3-(acetylthio)-3-methyl-1-oxopropyl]-cis-4-(4-pentenylthio)-L-proline is hydrolyzed with an aqueous ammonia solution

100 according to the procedure of Example 4 to yield 1-(D-3-mercaptoproline).

Example 23

(trans)-1-[D-3-(Acetylthio)-2-methyl-1-oxopropyl]-4-methoxy-L-proline, methyl ester

A solution of the material from Example 3 in ether is treated with a slight excess of diazomethane. After standing at room temperature, the solvent is evaporated to give (trans)-1-[D-3-(acetylthio)-2-

110 methyl-1-oxopropyl]-4-methoxy-L-proline, methyl ester.

Similarly, by employing the cis material from Example 7 in this procedure one obtains (cis)-1-[D-3-(acetylthio)-2-methyl-1-oxopropyl]-4-

115 methoxy-L-proline, methyl ester.

Example 24

1,1'-(Dithiodi-(1-D-3-mercaptopropane)-2-methyl-1-oxopropyl)-bis-[trans]-4-methoxy-L-proline

A solution of the material from Example 4 is dissolved in ethanol, stirred and treated with a solution of one equivalent of iodine in ethanol. The pH of the solution is maintained at 6-7 by the addition of N-sodium hydroxide solution. The solvent is evaporated and the residue extracted with ethyl acetate.

125 After drying over MgSO₄, the solution is filtered and the solvent is removed to give 1,1'-(dithiodi-(1-D-3-mercaptopropane)-2-methyl-1-oxopropyl)-bis-[trans]-4-methoxy-L-proline.

Similarly, by employing the cis material from

130 Example 8 in this procedure one obtains 1,1'-(

[dithiodi - (1 - D - 3 - mercapto - 2 - methyl - 1 - oxopropyl)] - bis - [(cis - 4 - methoxy - L - proline].

Example 25

Sodium salt of (trans) - 4 - methoxy - 1 - (D - 3 - 5 mercapto - 2 - methyl - 1 - oxopropyl) - L - proline

A solution of 2.5 g. of material from Example 4 in 25 ml. of water is treated with 0.84 g. of sodium bicarbonate. The solution is freeze-dried to give the sodium salt of (trans) - 4 - methoxy - 1 - (D - 3 - mercapto - 2 - methyl - 1 - oxopropyl) - L - proline.

Similarly, by employing the cis material from Example 8 in this procedure one obtains the sodium salt of (cis) - 4 - methoxy - 1 - (D - 3 - mercapto - 2 - methyl - 1 - oxopropyl) - L - proline.

15 *Example 26*

1 - (4 - Mercapto - 1 - oxobutyl) - cis - 4 - methoxy - L - proline

a) (cis) - 1 - (4 - Acetylthio - 1 - oxobutyl) - 4 - methoxy - L - proline

20 Interaction of an equivalent quantity of (cis) - 4 - methoxy - L - proline with 4 - acetylthiobutyroyl chloride according to the procedure described in Example 1 gives (cis) - 1 - (4 - acetylthio - 1 - oxobutyl) - 4 - methoxy - L - proline.

25 b) 1 - (4 - Mercapto - 1 - oxobutyl) - cis - 4 - methoxy - L - proline

Hydrolysis of (cis) - 1 - (4 - acetylthio - 1 - oxobutyl) - 4 - methoxy - L - proline with an aqueous ammonia solution according to the procedure of Example 2

30 yields 1 - (4 - mercapto - 1 - oxobutyl) - cis - 4 - methoxy - L - proline.

Example 27

1 - (L - 3 - Mercapto - 2 - ethyl - 1 - oxopropyl) - trans - 4 - methoxy - D - proline

35 a) (trans) - 1 - [L - (3 - Acetylthio) - 2 - ethyl - 1 - oxopropyl] - 4 - methoxy - D - proline

Utilizing the procedure of Example 1 but substituting (trans) - 4 - hydroxy - D - proline for the (trans) - 4 - hydroxy - L - proline in part (a) the (trans) - 4 - 40 methoxy - D - proline is obtained. By interacting the latter compound with L - (3 - acetylthio) - 2 - ethyl-propionyl chloride according to the procedure of Example 3, (trans) - 1 - [L - (3 - acetylthio) - 2 - ethyl - 1 - oxopropyl] - 4 - methoxy - D - proline is obtained.

45 b) 1 - (L - 3 - Mercapto - 2 - ethyl - 1 - oxopropyl) - trans - 4 - methoxy - D - proline

Hydrolysis of (trans) - 1 - [L - (3 - acetylthio) - 2 - ethyl - 1 - oxopropyl] - 4 - methoxy - D - proline with an aqueous ammonia solution according to the procedure of Example 4 gives 1 - (L - 3 - mercapto - 2 - ethyl - 1 - oxopropyl) - trans - 4 - methoxy - D - proline.

Example 28

1 - (2 - Mercapto - 1 - oxoethyl) - trans - 4 - methoxy - L - proline

55 a) (trans) - 1 - (2 - Acetylthio - 1 - oxoethyl) - 4 - methoxy - L - proline

Utilizing the procedure of Example 1, but substituting 60 2 - acetylthioacetyl chloride for the 3 - acetyl-thiopropionyl chloride, (trans) - 1 - (2 - acetylthio - 1 - oxoethyl) - 4 - methoxy - L - proline is obtained.

b) 1 - (2 - Mercapto - 1 - oxoethyl) - trans - 4 - methoxy - L - proline

Hydrolysis of trans - 1 - (2 - acetylthio - 1 - 65 oxoethyl) - 4 - methoxy - L - proline with an aqueous

ammonia solution according to the procedure of Example 2 yields 1 - (2 - mercapto - 1 - oxoethyl) - trans - 4 - methoxy - L - proline.

Example 29

70 1 - (2 - Mercapto - 1 - oxoethyl) - cis - 4 - methoxy - L - proline

a) (cis) - 1 - (2 - Acetylthio - 1 - oxoethyl) - 4 - methoxy - L - proline

Following the procedure of Example 28, cis - 4 - methoxy - L - proline is treated with a solution of 2 - acetylthioacetyl chloride in the presence of sodium carbonate to yield (cis) - 1 - (2 - acetylthio - 1 - oxoethyl) - 4 - methoxy - L - proline.

b) 1 - (2 - Mercapto - 1 - oxoethyl) - cis - 4 - methoxy - L - proline

Hydrolysis of (cis) - 1 - (2 - acetylthio - 1 - oxoethyl) - 4 - methoxy - L - proline with an aqueous ammonia solution yields 1 - (2 - mercapto - 1 - oxoethyl) - cis - 4 - methoxy - L - proline.

85 *Example 30*

1 - (2 - Mercapto - 1 - oxoethyl) - cis - 4 - methylthio - L - proline

a) (cis) - 1 - (2 - Acetylthio - 1 - oxoethyl) - 4 - methylthio - L - proline

90 Interaction of equivalent quantities of (cis) - 4 - methylthio - L - proline [J. Amer. Chem. Soc., 79, 185 (1957)] and 2 - acetylthioacetyl chloride according to the procedure described in Example 1, yields (cis) - 1 - (2 - acetylthio - 1 - oxoethyl) - 4 - methylthio - L - proline.

95 b) 1 - (2 - Mercapto - 1 - oxoethyl) - cis - 4 - methylthio - L - proline

Hydrolysis of (cis) - 1 - (2 - acetylthio - 1 - oxoethyl) - 4 - methylthio - L - proline with an aqueous

100 ammonia solution according to the procedure of Example 2 yields 1 - (2 - mercapto - 1 - oxoethyl) - cis - 4 - methylthio - L - proline.

Example 31

1 - (D - 3 - Mercapto - 3 - methyl - 1 - oxopropyl) - cis - 4 - (4 - pentynylthio) - L - proline

a) (cis) - 1 - [D - 3 - (Acetylthio) - 3 - methyl - 1 - oxopropyl] - 4 - (4 - pentynylthio) - L - proline

Interaction of (cis) - N - acetyl - 4 - mercapto - L - proline with 5 - chloro - 1 - pentyne in acetone in the presence of silver oxide according to the procedure of Example 1, part b, gives (cis) - N - acetyl - 4 - (4 - pentynylthio) - L - proline, 4 - pentynyl ester. The latter is then hydrolyzed as described in Example 1, part c, to give the (cis) - N - acetyl - 4 - (4 - pentynyl-

115 thio) - L - proline. Interaction of this compound with an equivalent quantity of D - 3 - (acetylthio) - 3 - methyl - propionyl chloride according to the procedure of Example 3 gives (cis) - 1 - [D - 3 - (acetylthio) - 3 - methyl - 1 - oxopropyl] - 4 - (4 - pentynylthio) - L - proline.

120 b) 1 - (D - 3 - Mercapto - 3 - methyl - 1 - oxopropyl) - cis - 4 - (4 - pentynylthio) - L - proline

Hydrolysis of (cis) - 1 - [D - 3 - (acetylthio) - 3 - methyl - 1 - oxopropyl] - 4 - (4 - pentynylthio) - L -

125 proline with an aqueous ammonia solution according to the procedure of Example 4 gives 1 - (D - 3 - mercapto - 3 - methyl - 1 - oxopropyl) - cis - 4 - (4 - pentynylthio) - L - proline.

Example 32

130 (cis) - 4 - Benzylthio - 1 - (D,L - 3 - mercapto - 3 - ethyl -

1 - oxopropyl] - L - proline

a) (cis) - 1 - [D,L - 3 - (Acetylthio) - 3 - ethyl - 1 - oxopropyl] - 4 - benzylthio - L - proline

Interaction of (cis) - N - acetyl - 4 - mercapto - L -

5 proline with benzyl chloride in acetone in the presence of silver oxide according to the procedure of Example 1, part b, gives (cis) - N - acetyl - 4 - benzylthio - L - proline, benzyl ester. The latter is hydrolyzed as described in Example 1, part c, to give (cis) - 10 N - acetyl - 4 - benzylthio - L - proline. Interaction of this compound with an equivalent amount of D,L - 3 - (acetylthio)valeroyl chloride according to the procedure of Example 3 gives (cis) - 1 - [D,L - 3 - (acetylthio) - 3 - ethyl - 1 - oxopropyl] - 4 - (benzylthio) - L - 15 proline.

b) (cis) - 4 - Benzylthio - 1 - (D,L - 3 - mercapto - 3 - ethyl - 1 - oxopropyl) - L - proline

Hydrolysis of (cis) - 1 - [D,L - 3 - (acetylthio) - 3 - ethyl - 1 - oxopropyl] - 4 - (benzylthio) - L - proline

20 with an aqueous ammonia solution according to the procedure of Example 4 gives (cis) - 4 - benzylthio - 1 - (D,L - 3 - mercapto - 3 - ethyl - 1 - oxopropyl) - L - proline.

Example 33**25 1,1' - [Dithiodi - (1 - D - 3 - mercapto - 2 - methyl - 1 - oxopropyl)] - bis[(trans) - 4 - methylthio - L - proline]**

The product of Example 21 is treated with iodine in ethanol according to the procedure described in Example 24 to give 1,1' - [dithiodi - (1 - D - 3 - mercapto - 2 - methyl - 1 - oxopropyl)] bis[(trans) - 4 - methylthio - L - proline].

Example 34**(cis) - 1 - [D - 3 - (Benzoylthio) - 2 - methyl - 1 - oxopropyl] - 4 - methylthio - L - proline**

35 Interaction of equivalent quantities of (cis) - 4 - methylthio - L - proline (J. Amer. Chem. Soc., 79, 185 (1957)) and D - 3 - (benzoylthio) - 2 - methyl - propionyl chloride according to the procedure of Example 3 gives (cis) - 1 - [D - 3 - (benzoylthio) - 2 - methyl - 40 1 - oxopropyl] - 4 - methylthio - L - proline.

Example 35**(trans) - 1 - [D - 3 - (Phenylacetyl) - 2 - methyl - 1 - oxopropyl] - 4 - methylthio - L - proline**

45 Interaction of equivalent quantities of (trans) - 4 - methylthio - L - proline (J. Amer. Chem. Soc., 79, 185 (1957)) and D - 3 - (phenylacetylthio) - 2 - methyl - propionyl chloride according to the procedure of Example 3 gives (trans) - 1 - [D - 3 - (phenylacetyl) - 2 - methyl - 1 - oxopropyl] - 4 - methylthio - L - proline.

Example 36**(cis) - 4 - (4 - Fluorophenoxy) - 1 - (D - 3 - mercapto - 2 - methyl - 1 - oxopropyl) - L - proline****a) (cis) - 4 - (4 - Fluorophenoxy) - L - proline**

To a solution of 8.4 g. (0.024 mole) of N - carbobenzoxy - trans - 4 - hydroxy - L - proline, benzyl ester [Baer et al., Can. J. Biochem. & Phys., 37, 583 (1959)], 4.0 g. (0.036 mole) of 4 - fluorophenol and 9.27 g. (0.036 mole) of triphenylphosphine in 75 ml. of dry tetrahydrofuran there is added dropwise over 60 one hour 6.2 g. (0.036 mole) of diethylazodicarboxylate in 25 ml. of tetrahydrofuran. The solution is allowed to stir overnight at room temperature. The mixture is evaporated to dryness and 100 ml. of ether is added to the residue. A precipitate of 65 triphenylphosphine and diethylazodicarboxylate is

filtered off. Column chromatography (silica gel) separates out 8.1 g. of a mixture containing about 70% N - carbobenzoxy - cis - 4 - (4 - fluorophenoxy) - L - proline, benzyl ester.

70 A solution of 7.5 g. of the above benzyl ester containing mixture is hydrogenated at 1 atmosphere (room temperature) with 0.8 g. of 10% Pd/C. A white precipitate forms during the reaction. After uptake of hydrogen ceases, the mixture is filtered and the filter

75 cake leached with three 125 ml. portions of hot methanol. The methanol solution is evaporated to dryness to yield 3.2 g. of cis - 4 - (4 - fluorophenoxy) - L - proline; m.p. 235-236°.

Anal. Calc'd. for $C_{11}H_{12}FNO_3 \cdot 1/3 H_2O$: C, 57.14; H,

80 5.48; N, 6.06; F, 8.22

Found: C, 56.94; H, 5.19; N, 5.94; F, 7.97.

b) 1 - [D - 3 - (Acetylthio) - 2 - methyl - 1 - oxopropyl] - cis - 4 - (4 - fluorophenoxy) - L - proline

To a suspension of 2.25 g. (0.01 mole) of cis - 4 - (4

85 - fluorophenoxy) - L - proline in 125 ml. of dry pyridine at room temperature there is added 1.0 g. (0.01 mole) of triethylamine and 2 g. (0.011 mole) of D - 3 - (acetylthio) - 2 - methylpropionyl - chloride. After stirring overnight at room temperature the

90 mixture is evaporated to dryness. The residue is taken up in water, covered with ether and acidified with 10% hydrochloric acid. The water layer is repeatedly extracted with ether, the ether layers are combined, washed with water, dried (Na_2SO_4) and 95 evaporated to dryness to yield 3.9 g. of residue. The residue is chromatographed on 250 ml. of silica gel with ether to yield a fraction containing the desired product. The column is washed with methanol and the methanol washing is rechromatographed on a

100 short silica gel column to yield more product. This procedure is repeated once more to yield a total of 1.2 g. of pure 1 - [D - 3 - (acetylthio) - 2 - methyl - 1 - oxopropyl] - cis - 4 - (4 - fluorophenoxy) - L - proline.

c) (cis) - 4 - (4 - Fluorophenoxy) - 1 - (D - 3 - mercapto -**105 2 - methyl - 1 - oxopropyl) - L - proline**

1.2 g. of 1 - [D - 3 - (acetylthio) - 2 - methyl - 1 - oxopropyl] - cis - 4 - (4 - fluorophenoxy) - L - proline

is dissolved in 25 ml. of methanol and treated with 5 ml. of concentrated ammonia under argon for 40

110 minutes. The volatiles are stripped off and the residue is covered with ethyl acetate. The water layer is acidified with 10% hydrochloric acid. The layers are separated and the acid layer is washed with ethyl acetate. The combined organic layers are stripped 115 with water (2x), saturated sodium chloride solution (2x), and dried (Na_2SO_4). The solvent is stripped off to yield 1.1 g. of a solid foam residue of cis - 4 - (4 - fluorophenoxy) - 1 - (D - 3 - mercapto - 2 - methyl - 1 - oxopropyl) - L - proline.

120 Anal. Calc'd. for $C_{15}H_{18}FNO_4 \cdot 1/3 H_2O$: C, 54.05; H,

5.70; N, 4.20; F, 5.70; S, 9.60

Found: C, 54.04; H, 5.71; N, 4.05; F, 5.40; S, 9.61.

Thin layer chromatography showed the product at R, 0.30 as detected by UV-light, SH reagent (yellow

125 spot) and vanillin (yellow spot). $[\alpha]^{25}_D = -80^\circ$ (c, 1% in chloroform).

Example 37**1 - (3 - Mercapto - 1 - oxopropyl) - cis - 4 - phenoxy - L - proline****130 a) (cis) - 4 - Phenoxy - L - proline**

Following the procedure of Example 36(a) but substituting an equivalent amount of phenol for the 4-fluorophenol one obtains (cis)-4-phenoxy-L-proline.

5 b) 1-[3-(Acetylthio)-1-oxopropyl]-cis-4-phenoxy-L-proline

The (cis)-4-phenoxy-L-proline is reacted with a solution of 3-acetylthiopropionyl chloride in the presence of sodium carbonate according to the procedure of Example 1 to yield 1-[3-(acetylthio)-1-oxopropyl]-cis-4-phenoxy-L-proline.

c) 1-(3-Mercapto-1-oxopropyl)-cis-4-phenoxy-L-proline

Hydrolysis of 1-[3-(acetylthio)-1-oxopropyl]-

15 cis-4-phenoxy-L-proline with an aqueous ammonia solution according to the procedure of Example 2 yields 1-(3-mercaptopro-1-oxopropyl)-cis-4-phenoxy-L-proline.

Example 38

20 1-(D-3-Mercapto-2-methyl-1-oxopropyl)-cis-4-(4-methyl-benzyloxy)-L-proline

Following the procedure of Example 36 but substituting an equivalent amount of 4-methylbenzyl alcohol for the 4-fluorophenol in part (a) one

25 obtains 1-(D-3-mercaptopro-2-methyl-1-oxopropyl)-cis-4-(4-methyl-benzyloxy)-L-proline.

Example 39

1-(D-3-Mercapto-2-methyl-1-oxopropyl)-cis-4-phenethyloxy-L-proline

Following the procedure of Example 36 but substituting an equivalent amount of phenethyl alcohol for the 4-fluorophenol in part (a) one obtains 1-(D-3-mercaptopro-2-methyl-1-oxopropyl)-cis-4-phenethyloxy-L-proline.

35 Example 40

1-(D-3-Mercapto-2-methyl-1-oxopropyl)-cis-4-(3-methyl-thiophenoxy)-L-proline

Following the procedure of Example 36 but substituting an equivalent amount of 3-methyl-

40 thiophenol for the 4-fluorophenol in part (a) one obtains 1-(D-3-mercaptopro-2-methyl-1-oxopropyl)-cis-4-(3-methylthiophenoxy)-L-proline.

Example 41

(cis)-4-(4-Chlorophenoxy)-1-(D-3-mercaptopro-2-methyl-1-oxopropyl)-L-proline

Following the procedure of Example 36 but substituting an equivalent amount of 4-chlorophenol for the 4-fluorophenol in part (a) one obtains (cis)-4-(4-chlorophenoxy)-1-(D-3-mercaptopro-2-methyl

50 -1-oxopropyl)-L-proline.

Example 42

1-(2-Mercapto-1-oxoethyl)-cis-4-(4-methoxyphenoxy)-L-proline

a) (cis)-4-(4-Methoxyphenoxy)-L-proline

Following the procedure of Example 36 (a) but substituting an equivalent amount of 4-methoxyphenol for the 4-fluorophenol one obtains (cis)-4-(4-methoxyphenoxy)-L-proline.

b) 1-[2-(Acetylthio)-1-oxoethyl]-cis-4-(4-

60 methoxyphenoxy)-L-proline

The (cis)-4-(4-methoxyphenoxy)-L-proline is reacted with a solution of 2-acetylthioacetyl chloride according to the procedure of Example 1, part (d), to yield 1-[2-(acetylthio)-1-oxoethyl]-cis-

65 -4-(4-methoxyphenoxy)-L-proline.

c) 1-(2-Mercapto-1-oxoethyl)-cis-4-(4-methoxyphenoxy)-L-proline

Hydrolysis of 1-[2-(acetylthio)-1-oxoethyl]-cis-4-(4-methoxyphenoxy)-L-proline with an aqueous ammonia solution according to the procedure of Example 2 yields 1-(2-mercaptopro-1-oxoethyl)-cis-4-(4-methoxyphenoxy)-L-proline.

Example 43

(cis)-4-(4-Fluorophenylthio)-1-(D-3-mercaptopro-2-methyl-1-oxopropyl)-L-proline

Following the procedure of Example 36 but substituting an equivalent amount of 4-fluorophenylmercaptan for the 4-fluorophenol in part (a) one obtains (cis)-4-(4-fluorophenylthio)-1-(D-3-

80 mercapto-2-methyl-1-oxopropyl)-L-proline

Example 44

1-(D-3-Mercapto-2-methyl-1-oxopropyl)-cis-4-phenylthio-L-proline

a) N-Carbobenzyloxy-cis-4-phenylthio-L-proline, methyl ester

Sodium metal (0.85 g., 0.037 mole) is dissolved in 40 ml. of absolute ethanol. To this there is added with stirring 3.7 ml. (0.036 mole) of thiophenol followed by 7.5 g. (0.017 mole) of N-carbobenzyloxy-trans-4-tosyloxy-L-proline, methyl ester [J. Am. Chem. Soc., 79, 191 (1957)]. The latter gradually goes into solution but soon thereafter a solid product separates. After stirring for 4 hours and standing overnight at room temperature, the bulk of the

95 ethanol is removed on a rotary evaporator. The mostly solid residue is stirred with 120 ml. of dichloromethane and 60 ml. of water. The layers are separated (some methanol is added to help break up emulsions) and the aqueous phase is extracted with

100 additional dichloromethane (2 × 60 ml.). The combined organic phase are washed with 100 ml. of saturated sodium chloride solution, dried ($MgSO_4$), and the solvent evaporated to give 6.5 g. (100%) of N-carbobenzyloxy-cis-4-phenylthio-L-proline,

105 methyl ester as a pale yellow viscous oil.

b) N-Carbobenzyloxy-cis-4-phenylthio-L-proline

The methyl ester product from part (a) (6.5 g., 0.017 mole) is dissolved in 55 ml. of methanol, treated portionwise at -1° to 4° with 13 ml. (0.026 mole) of 2N sodium hydroxide, stirred at 0° for one hour, and kept at room temperature for approximately 16 hours. After removing about half of the solvent on a rotary evaporator, the cooled solution is diluted with

110 100 ml. of water, washed with 60 ml. of ether (wash discarded), layered over with 70 ml. of ethyl acetate, stirred, cooled, and acidified with 4.8 ml. of 1:1 hydrochloric acid. After separating, the aqueous phase is extracted with additional ethyl acetate (3 × 40 ml.)

120 and the combined organic layers are dried ($MgSO_4$) and evaporated to give 5.9 g. of a light yellow viscous oil. The latter is dissolved in 30 ml. of ethanol, treated with 1.9 g. of cyclohexylamine in 3 ml. of ethanol, and diluted to 330 ml. with ether. On seeding, the crystalline cyclohexylamine salt separates.

125 The latter, after cooling for approximately 16 hours, weighs 5.3 g.; m.p. 148-151° (s. 135°). This material is combined with 1.5 g. of identical product from a previous experiment, stirred with 200 ml. of boiling acetonitrile, and cooled to yield 6.3 g. of colorless

cyclohexylamine salt; m.p. 152-155° (s. 137°) [α]₂₅^b -24° (c, 1% in ethanol).

This cyclohexylamine salt is suspended in 25 ml. of ethyl acetate, stirred, and treated with 25 ml. of N 5 hydrochloric acid. When two clear layers are obtained, they are separated and the aqueous phase is extracted with additional ethyl acetate (3 × 25 ml.). The combined organic layers are dried ($MgSO_4$) and the solvent evaporated to give 5.0 g. (65%) of N - 10 carbobenzyloxy - cis - 4 - phenylthio - L - proline as a nearly colorless, very viscous syrup.

c) (cis) - 4 - Phenylthio - L - proline hydrobromide

N - Carbobenzyloxy - cis - 4 - phenylthio - L - proline (4.9 g., 0.014 mole) is treated with 25 ml. of hydrogen bromide in acetic acid (30-32%), stoppered loosely, and stirred magnetically. After one hour the orange-yellow solution is diluted to 250 ml. with ether to precipitate the product as a heavy oil which gradually crystallizes on seeding, rubbing and cooling. After stirring in an ice-bath for one hour, the material is filtered under nitrogen, washed with ether, suspended in fresh ether, cooled for approximately 16 hours, and filtered again to give 3.2 g. (77%) of colorless solid (cis) - 4 - phenylthio - L - 25 proline hydrobromide; m.p. 106-109° (s. 99°), [α]₂₅^b -3° (c, 1% in methanol).

d) 1 - [D - 3 - (Acetylthio) - 2 - methyl - 1 - oxopropyl] - cis - 4 - phenylthio - L - proline

Interaction of 3.0 g. (0.0094 mole) of (cis) - 4 - 30 phenylthio - L - proline hydrobromide and 2.0 g. (0.011 mole) of D - 3 - acetylthio - 2 - methylpropionyl chloride in 25 ml. of water as described in Example 1, part (d) (using approximately 15 ml. of 20% sodium carbonate solution to maintain the pH at 8.0 to 8.4), 35 yields 3.8 g. of a pale yellow viscous oil. The dicyclohexylamine salt (prepared in 30 ml. of ethyl acetate employing 1.8 g. of dicyclohexylamine) weighs 2.9 g. (isolated in two crops); m.p. 184-188° (s. 180°). Following trituration with 15 ml. of acetonitrile, one 40 obtains 2.4 g. of colorless solid dicyclohexylamine salt; m.p. 184-186° (s. 180°), [α]₂₅^b -75° (c, 1% in ethanol).

This dicyclohexylamine salt is treated with 30 ml. of 10% potassium bisulfate and extracted into ethyl 45 acetate as described in Example 1 to yield 2.0 g. (59%) of glass-like 1 - [D - 3 - (acetylthio) - 2 - methyl - 1 - oxopropyl] - cis - 4 - phenylthio - L - proline. e) 1 - (D - 3 - Mercapto - 2 - methyl - 1 - oxopropyl) - cis - 4 - phenylthio - L - proline

50 The 1 - [D - 3 - (acetylthio) - 2 - methyl - 1 - oxopropyl] - cis - 4 - phenylthio - L - proline (2.0 g., 0.0042 mole) is treated with 3.5 ml. of concentrated ammonia in 8.5 ml. of water according to the procedure of Example 2 (the solid ammonium salt of the 55 product separates from the reaction mixture) to give 1.35 g. (100%) of viscous syrupy 1 - (D - 3 - mercapto - 2 - methyl - 1 - oxopropyl) - cis - 4 - phenylthio - L - proline, [α]₂₅^b -43° (c, 1% in ethanol).

Example 45

60 1 - (D - 3 - Mercapto - 2 - methyl - 1 - oxopropyl) - cis - 4 - (4 - chlorophenylthio) - L - proline

Following the procedure of Example 44 but substituting an equivalent amount of 4 - chlorophenylmercaptan for the thiophenol in part (a) one obtains 65 1 - (D - 3 - mercapto - 2 - methyl - 1 - oxopropyl) - cis -

4 - (4 - chlorophenylthio) - L - proline.

Example 46

1 - (D - 3 - Mercapto - 2 - methyl - 1 - oxopropyl) - cis - 4 - (3 - trifluoromethylphenylthio) - L - proline

70 Following the procedure of Example 44 but substituting an equivalent amount of 3 - trifluoromethylphenylmercaptan for the thiophenol in part (a) one obtains 1 - (D - 3 - mercapto - 2 - methyl - 1 - oxopropyl) - cis - (3 - trifluoromethylphenylthio) - L - proline.

Example 47

(cis) - 4 - (4 - Hydroxyphenylthio) - 1 - (3 - mercapto - 1 - oxopropyl) - L - proline

a) (cis) - 4 - (4 - Acetoxyphenylthio) - L - proline

80 hydrobromide

Following the procedure of Example 44 (a) to (c) but substituting an equivalent amount of 4 - acetoxy - phenylmercaptan for the thiophenol in part (a) one obtains (cis) - 4 - (4 - acetoxyphenylthio) - L - proline hydrobromide.

b) (cis) - 4 - (4 - Acetoxyphenylthio) - 1 - [3 - (acetylthio) - 1 - oxopropyl] - L - proline

Interaction of (cis) - 4 - (4 - acetoxyphenylthio) - L - proline hydrobromide and 3 - acetylthiopropionyl

90 chloride according to the procedure described in Example 1, part (d) yields (cis) - 4 - (4 - acetylthiophenylthio) - 1 - [3 - acetylthio) - 1 - oxopropyl] - L - proline.

c) (cis) - 4 - (4 - Hydroxyphenylthio) - 1 - (3 - mercapto - 1

95 - oxopropyl) - L - proline

Hydrolysis of (cis) - 4 - (4 - acetoxyphenylthio) - 1 - [3 - (acetylthio) - 1 - oxopropyl] - L - proline with an aqueous ammonia solution according to the procedure of Example 2 yields (cis) - 4 - (4 - hydroxyphenylthio) - 1 - (3 - mercapto - 1 - oxopropyl) - L - proline.

Example 48

(cis) - 4 - Benzyloxy - 1 - (D - 3 - mercapto - 2 - methyl - 1 - oxopropyl) - L - proline

105 Following the procedure of Example 44 but substituting an equivalent amount of benzyl alcohol for the thiophenol in part (a) one obtains (cis) - 4 - benzyloxy - 1 - (D - 3 - mercapto - 2 - ethyl - 1 - oxopropyl) - L - proline.

110 Example 49

(trans) - 1 - (3 - Mercapto - 1 - oxopropyl) - 3 - methylthio - D,L - proline

a) 1,2 - Dehydropoline, t - butyl ester

To a stirred solution of 34.2 g. (0.20 mole) of pro-

115 line t - butyl ester in 600 ml. of ether at -5° to 0° is added dropwise over ten minutes 21.7 g. (23.9 ml., 0.20 mole) of freshly prepared t - butyl hypochlorite [Org. Syn., Coll. Vol. V, 184 (1973)]. During the addition, the temperature is maintained at -5° to 0°. After

120 the addition is complete, the solution is stirred at this temperature for an additional five minutes.

To the vigorously stirred solution is added rapidly (~3-5 min.) a solution of 7.8 g. (0.20 mole) of potassium in freshly distilled dry (CaH_2) t - butanol. After

125 the addition, the temperature of the reaction mixture is about 18°. The reaction vessel is removed from the cooling bath and stirred for thirty minutes. The reaction mixture is filtered through Celite (diatomaceous earth) and the filtrate concentrated *in vacuo*. The residue is taken up in ether and washed with several

130

- portions of water. The ether solution is dried and concentrated *in vacuo* to 31.6 g. of yellow liquid. A trace of hydroquinone is added and the crude product distilled, affording 22.4 g. of 1,2 - dehydrop-5 roline, t - butyl ester (66%), b.p. 60-62°/0.1 mm.
- b) 1 - Benzyloxycarbonyl - 4,5 - dihydro - 1H - pyrrole - 2 - carboxylic acid, t - butyl ester
- A solution of 16.9 g. (0.1 mole) of 1,2 - dehydروline, t - butyl ester in 70 ml. of dichloromethane is cooled to -10° under argon. A solution of freshly distilled benzylchloroformate (14.2 ml., 0.1 mole, b.p. 62-64° (0.4 mm)) in 70 ml. of dichloromethane is added dropwise over a period of 30 minutes. After stirring in the cold for another 30 minutes a solution of 15.22 g. (0.1 mole) of 1,5 - diazabicyclo - [5.4.0]undec - 5 - ene in 70 ml. of dichloromethane is added over a period of 20 minutes. The cooling bath is then removed and the mixture is stirred at room temperature for one hour. After washing twice with cold dilute hydrochloric acid and once with saturated sodium carbonate solution, the solution is dried *in vacuo* to yield 18.2 g. (60%) of 1 - benzyloxycarbonyl - 4,5 - dihydro - 1H - pyrrole - 2 - carboxylic acid, t - butyl ester as a pale yellow oil.
- c) (trans) - 1 - Benzyloxycarbonyl - 3 - methylthio - D,L - proline, t - butyl ester
- A solution of 18.2 g. (0.06 mole) of 1 - benzyloxycarbonyl - 4,5 - dihydro - 1H - pyrrole - 2 - carboxylic acid, t - butyl ester in 180 ml. of dry methanol is treated with 3.24 g. (0.06 mole) of sodium methoxide and cooled in an ice-bath. Methanethiol is bubbled into the solution slowly for 30 minutes. The mixture is stirred overnight under argon at room temperature. Dilute aqueous acetic acid is added until the solution is slightly acidic. Argon is bubbled through the solution for one hour before it is taken to near dryness *in vacuo*. Ethyl acetate is added and the solution is washed twice with saturated sodium carbonate solution, dried and freed of solvent *in vacuo* to yield 17 g. of yellow oil. This oil is chromatographed using 300 g. of silica gel and petroleum ether: ether (4:1) to yield 11.1 g. of (trans) - 1 - benzyloxycarbonyl - 3 - methylthio - D,L - proline as a colourless oil.
- d) (trans) - 3 - Methylthio - D,L - proline
- 8.4 g. (0.024 mole) of (trans) - 1 - benzyloxy - carbonyl - 3 - methylthio - D,L - proline are treated with 45 ml. of 4N hydrobromic acid in acetic acid. After stirring for one hour at room temperature the solution is dried *in vacuo*. A small amount of water is added and this is washed twice with ether. The aqueous solution is applied to a column containing 300 ml. of an ion-exchange resin and water is passed through until the eluate is no longer strongly acidic. The product is then eluted with pH 6.5 (aqueous pyridine acetate) buffer. Fractions positive to ninhydrin are combined and lyophilized to give 3.4 g. (88%) of white fluff. A small sample of this material is crystallized from methanol to give (trans) - 3 - methylthio - D,L - proline; m.p. 196-200° (dec.) (s. 192).
- Anal. Calc'd. for C₁₁H₁₇O₂NS: C, 44.70; H, 6.88; N, 8.69; S, 19.89
- Found: C, 44.53; H, 7.10; N, 8.61; S, 19.95.
- e) (trans) - 1 - [3 - (Acetylthio) - 1 - oxopropyl] - 3 - methylthio - D,L - proline
- 3.05 g. (0.019 mole) of (trans) - 3 - methylthio - D,L - proline is dissolved in 19 ml. of 1N sodium carbonate and diluted with 10 ml. of water. The solution is cooled in an ice-bath and while stirring rapidly a solution of 3 - acetylthiopropionyl chloride in 20 ml. of ether is added. The pH is maintained at 8 by adding 1N sodium carbonate. At the end of 30 minutes the pH is holding constant and 45 ml. of sodium carbonate solution had been added. The layers are separated and the aqueous layer is washed once with ether. The aqueous layer is then acidified with 10% potassium bisulfate solution and the product is extracted into ethyl acetate, dried and freed of solvent *in vacuo* to give 5.2 g. of oil. The material is chromatographed on 150 g. silica gel using ethyl acetate for elution. 3.85 g. of somewhat crude (trans) - 1 - [3 - (acetylthio) - 1 - oxopropyl] - 3 - methylthio - D,L - proline are obtained.
- A small sample is dissolved in ether and converted to the dicyclohexylamine salt which is the recrystallized from ethyl acetate to give (trans) - 1 - [3 - (acetylthio) - 1 - oxopropyl] - 3 - methylthio - D,L - proline, dicyclohexylamine salt; m.p. 153-157°.
- Anal. Calc'd. for C₁₁H₁₇O₂NS · (C₆H₁₁)₂NH: C, 58.44; H, 8.53; N, 5.83; S, 13.57
- Found: C, 58.77; H, 8.57; N, 5.68; S, 13.74.
- f) (trans) - 1 - (3 - Mercapto - 1 - oxopropyl) - 3 - methylthio - D,L - proline
- 2.05 g. (0.007 mole) of (trans) - 1 - [3 - (acetylthio) - 1 - oxopropyl] - 3 - methylthio - D,L - proline is cooled in an ice-bath under argon and treated with a cold argon saturated mixture of 7 ml. of water and 7 ml. of concentrated ammonia. After stirring for 30 minutes at 0° the solution is acidified with hydrochloric acid. The product is extracted into ethyl acetate, dried and freed of solvent *in vacuo* to give 1.85 g. of material which becomes partially crystalline on standing. Trituration with ether gives 1.0 g. (57%) of white crystalline product. Recrystallization from ethyl acetate (5 ml.) gives 0.85 g. of (trans) - 1 - (3 - mercapto - 1 - oxopropyl) - 3 - methylthio - D,L - proline; m.p. 89-93°.
- Anal. Calc'd. for C₉H₁₅O₂NS₂: C, 43.35; H, 6.06; N, 5.62; S, 25.72
- 110 Found: C, 43.35; H, 6.27; N, 5.54; S, 25.91.
- Example 50*
- (trans) - 1 - (D - 3 - Mercapto - 2 - methyl - 1 - oxopropyl) - 3 - ethylthio - D,L - proline
- a) (trans) - 3 - Ethylthio - D,L - proline
- 115 Following the general procedure of Example 49 (a) to (d) but substituting an equivalent amount of ethyl mercaptan for the methanethiol one obtains (trans) - 3 - ethyl - D,L - proline.
- b) (trans) - 1 - [D - 3 - (Acetylthio) - 2 - methyl - 1 - oxopropyl] - 3 - ethylthio - D,L - proline
- Interaction of the (trans) - 2 - ethylthio - D,L - proline and D - 3 - acetylthio - 2 - methylpropionyl chloride according to the procedure of Example 3 yields (trans) - 1 - [D - 3 - (acetylthio) - 2 - methyl - 1 - oxopropyl] - 3 - ethylthio - D,L - proline.
- c) (trans) - 1 - (D - 3 - Mercapto - 2 - methyl - 1 - oxopropyl) - 3 - ethylthio - D,L - proline
- Hydrolysis of (trans) - 1 - [D - 3 - (acetylthio) - 2 - methyl - 1 - oxopropyl] - 3 - ethylthio - D,L - proline
- 130 with an aqueous ammonia solution according to the

procedure of Example 4 yields (trans) - 1 - (D - 3 - mercapto - 2 - methyl - 1 - oxopropyl) - 3 - ethylthio - D,L - proline.

Example 51

5 (trans) - 1 - (3 - Mercapto - 1 - oxopropyl) - 3 - phenylthio - D,L - proline

Following the general procedure of Example 49 but substituting an equivalent amount of thiophenol for the methanethiol yields (trans) - 1 - (3 - mercapto - 1 - oxopropyl) - 3 - phenylthio - D,L - proline.

Example 52

(cis) - 4 - Methoxy - 1 - (D - 3 - mercapto - 2 - trifluoromethyl - 1 - oxopropyl) - L - proline

a) 3 - (4 - Methoxybenzyl)thio - 2 - trifluoromethylpropionyl chloride

A neat mixture of 1 - trifluoromethylacrylic acid (3.9 g.) and 4 - methoxybenzylthio (4.3 g.) is stirred at 100-110° for one hour. The mixture is allowed to cool to room temperature and the solid is recrystallized from cyclohexane to yield 3 - (4 - methoxybenzyl)thio - 2 - trifluoromethylpropanoic acid, m.p. 72-74°.

Treatment of this acid with thionyl chloride yields 3 - (4 - methoxybenzyl)thio - 2 - trifluoromethylpropionyl chloride.

25 b) (cis) - 4 - Methoxy - 1 - [D - 3 - (4 - methoxybenzyl)thio - 2 - trifluoromethyl - 1 - oxopropyl] - L - proline

The 3 - (4 - methoxybenzyl)thio - 2 - trifluoromethylpropionyl chloride is reacted with (cis) - 4 - methoxy - L - proline to yield (cis) - 4 - methoxy - 1 - [D - 3 - (4 - methoxybenzyl)thio - 2 - trifluoromethyl - 1 - oxopropyl] - L - proline.

c) (cis) - 4 - Methoxy - 1 - (D - 3 - mercapto - 2 - trifluoromethyl - 1 - oxopropyl) - L - proline

35 The (cis) - 4 - methoxy - 1 - [D - 3 - (4 - methoxybenzyl)thio - 2 - trifluoromethyl - 1 - oxopropyl] - L - proline is mixed with trifluoroacetic acid and anisole and stirred under nitrogen. The solvents are removed under vacuum to yield as a residue (cis) - 4 - 40 - methoxy - 1 - (D - 3 - mercapto - 2 - trifluoromethyl - 1 - oxopropyl) - L - proline.

Example 53

(trans) - 4 - Methoxy - 1 - (D - 3 - mercapto - 2 - trifluoromethyl - 1 - oxopropyl) - L - proline

45 Following the procedure of Example 52 but substituting (trans) - 4 - methoxy - L - proline for the cis isomer one obtains (trans) - 4 - methoxy - 1 - (D - 3 - mercapto - 2 - trifluoromethyl - 1 - oxopropyl) - L - proline.

Example 54

(trans) - 4 - Etheno - 1 - (D - 3 - mercapto - 2 - trifluoromethyl - 1 - oxopropyl) - L - proline

a) 3 - Acetylthio - 2 - trifluoromethylpropionyl chloride

55 A mixture of thiolacetic acid and 2 - (trifluoromethyl)acrylic acid is heated on a steam bath for one hour and then stored at room temperature for 18 hours. The reaction mixture is distilled in *vacuo* to give 3 - acetylthio - 2 - trifluoromethylpropanoic acid.

Treatment of this acid with thionyl chloride yields 3 - acetylthio - 2 - trifluoromethylpropionyl chloride.

b) (trans) - 4 - Etheno - 1 - [D - 3 - (acetylthio) - 2 - trifluoromethyl - 1 - oxopropyl] - L - proline

65 Reacting 3 - acetylthio - 2 - trifluoromethyl-

propionyl chloride with trans - 4 - ethoxy - L - proline according to the procedure of Example 3 yields (trans) - 4 - ethoxy - 1 - [D - 3 - (acetylthio) - 2 - trifluoromethyl - 1 - oxopropyl] - L - proline.

70 c) (trans) - 4 - Etheno - 1 - (D - 3 - mercapto - 2 - trifluoromethyl - 1 - oxopropyl) - L - proline

Treating (trans) - 4 - ethoxy - 1 - [D - 3 - (acetylthio) - 2 - trifluoromethyl - 1 - oxopropyl] - L - proline with an aqueous solution of ammonia according to the

75 procedure of Example 4 yields (trans) - 4 - ethoxy - 1 - (D - 3 - mercapto - 2 - trifluoromethyl - 1 - oxopropyl) - L - proline.

Example 55

1000 tablets each containing 100 mg. of 1 - (D - 3 - mercapto - 1 - oxopropyl) - trans - 4 - methoxy - L - proline, sodium salt, are produced from the following ingredients:

1 - (3 - mercapto - 1 - oxopropyl) - trans - 4 - methoxy - L - proline, sodium salt 100 g.

85 Corn starch 50 g.

Gelatin 7.5 g.

Avicel (microcrystalline cellulose) 25 g.

Magnesium stearate 2.5 g.

The 1 - (D - 3 - mercapto - 1 - oxopropyl) - trans - 4 -

90 methoxy - L - proline salt and corn starch are admixed with an aqueous solution of the gelatin. The mixture is dried and ground to a fine powder.

The Avicel and then the magnesium stearate are admixed with the granulation. This is then compressed

95 in a tablet press to form 1000 tablets each containing 100 mg. of active ingredient.

Example 56

Tablets each containing 200 mg. of 1 - (D - 3 - mercapto - 2 - methyl - 1 - oxopropyl) - trans - 4 - methoxy - L - proline are produced as described in Example 55.

Example 57

1000 tablets each containing 200 mg. of 1 - (D - 3 - mercapto - 2 - methyl - 1 - oxopropyl) - cis - 4 - methoxy - L - proline, sodium salt, are produced from the following ingredients:

1 - (D - 3 - mercapto - 2 - methyl - 1 - oxopropyl) - cis - 4 - methoxy - L - proline, sodium salt 50 g.

110 Lactose 25 g.

Avicel 38 g.

Corn starch 15 g.

Magnesium stearate 2 g.

The 1 - (D - 3 - mercapto - 2 - methyl - 1 - oxopropyl) -

115 - cis - 4 - methoxy - L - proline, sodium salt, lactose and Avicel are admixed, then blended with the corn starch. Magnesium stearate is added. The dry mixture is compressed in a tablet press to form 1000 130 mg. tablets each containing 50 mg. of active ingre-

120 dient. The tablets are coated with a solution of Methocel E 15 (methyl cellulose) including as a color a lake containing yellow #6.

Example 58

Two piece #1 gelatin capsules each containing 100 mg. of 1 - (D - 3 - mercapto - 2 - methyl - 1 - oxopropyl) - cis - 4 - methoxy - L - proline, sodium salt, are filled with a mixture of the following ingredients:

1 - (D - 3 - mercapto - 2 - methyl - 1 - oxopropyl) -

130 cis - 4 - methoxy - L - proline, sodium salt 100 mg.

Magnesium stearate 7 mg.
USP Lactose 193 mg.

Example 59

An injectable solution is produced as follows:

- 5 (trans) - 4 - methoxy - 1 - (D - 3 - mercapto - 2 - methyl - 1 - oxopropyl) - L - proline 500 g.
Methyl paraben 5 g.
Propyl paraben 1 g.
Sodium chloride 25 g.
10 Water for injection qs. 5 l

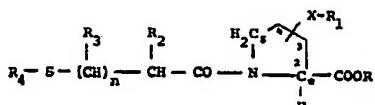
The active substance, preservatives and sodium chloride are dissolved in 3 liters of water for injection and then the volume is brought up to 5 liters. The solution is filtered through a sterile filter and aseptically filled into presterilized vials which are then closed with presterilized rubber closures. Each vial contains 5 ml. of solution in a concentration of 100 mg. of active ingredient per ml. of solution for injection.

15 The products of each example can be similarly formulated as in Examples 55-59.

CLAIMS

1. A compound of the formula

25



wherein the X-R₁ group is located at the 3 - or 4 - position of the proline ring;

X is oxygen or sulfur;

R is hydrogen or lower alkyl;

R₁ is lower alkyl, lower alkenyl, lower alkynyl, phenyl, substituted phenyl, phenyl-lower alkylene, 35 or substituted phenyl-lower alkylene;

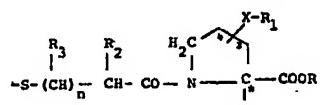
R₂ and R₃ are independently selected from hydrogen, lower alkyl and trifluoromethyl;

O

||

40 R₄ is hydrogen, R₅-C- or

45



R₅ is lower alkyl, phenyl, or phenyl-lower alkylene; and

n is 0, 1 or 2, or such a compound in salt form.

50 2. The compound of Claim 1 wherein X is oxygen.

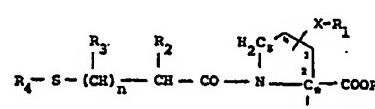
3. The compound of Claim 1 wherein X is sulfur.

4. The compound of Claim 1, 2 or 3 wherein R₄ is hydrogen.

55 5. The compound of Claim 1, 2, 3 or 4 wherein the proline ring is in the L - configuration.

6. A compound of the formula

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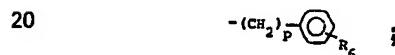
wherein the X-R₁ group is located at the 3 - or 4 - position of the proline ring;

X is oxygen or sulfur;
R is hydrogen;
R₁ is lower alkyl of 1 to 3 carbons or

- 70 $-(CH_2)_p-\text{C}_6\text{H}_4-\text{R}_6$
R₂ is hydrogen, methyl or trifluoromethyl;
R₃ is hydrogen;
R₄ is hydrogen, acetyl or benzoyl;
75 R₆ is hydrogen, methyl, methoxy, methylthio, chloro, fluoro, trifluoromethyl or hydroxy;
n is zero or one; and
p is zero, one or two, or such a compound in salt form.
- 80 7. The compound of Claim 6 wherein X is oxygen.
8. The compound of Claim 7 wherein R₄ is hydrogen; R₂ is hydrogen or methyl; and n is one.
9. The compound of Claim 8 wherein R₁ is lower alkyl of 1 to 3 carbons.
10. The compound of Claim 9 wherein the proline ring is in the L - configuration; when R₂ is other than hydrogen the asymmetric carbon to which R₂ is attached is in the D - configuration; and -O-R₁ group is at the 4-position of the proline ring and in the cis configuration.
11. The compound of Claim 10 wherein R₁ is methyl.
12. The compound of Claim 11, (cis) - 4 - methoxy - 1 - (D - 3 - mercapto - 2 - methyl - 1 - oxopropyl) - L - proline.
13. The compound of claim 11, (cis) - 4 - methoxy - 1 - (3 - mercapto - 1 - oxopropyl) - L - proline.
14. The compound of Claim 9 wherein the proline ring is in the L - configuration; when R₂ is other than hydrogen the asymmetric carbon to which R₂ is attached is in the D - configuration; and the -O-R₁ group is at the 4-position of the proline ring and in the trans configuration.
- 100 15. The compound of Claim 14 wherein R₁ is methyl.
16. The compound of Claim 15, (trans) - 4 - methoxy - 1 - (D - 3 - mercapto - 2 - methyl - 1 - oxopropyl) - L - proline.
- 110 17. The compound of Claim 15, trans - 4 - methoxy - 1 - (3 - mercapto - 1 - oxopropyl) - L - proline.
18. The compound of Claim 14 wherein R₁ is ethyl.
- 115 19. The compound of Claim 18, (trans) - 4 - ethoxy - 1 - (D - 3 - mercapto - 2 - methyl - 1 - oxopropyl) - L - proline.
20. The compound of Claim 14 wherein R₁ is n - propyl.
- 120 21. The compound of Claim 20, (trans) - 4 - propoxy - 1 - (D - 3 - mercapto - 2 - methyl - 1 - oxopropyl) - L - proline.
22. The compound of Claim 8 wherein R₁ is

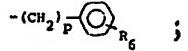
- 125 $-(CH_2)_p-\text{C}_6\text{H}_4-\text{R}_6$
R₆ is hydrogen, methyl, methoxy, methylthio, chloro, fluoro, trifluoromethyl or hydroxy; and p is zero, one or two.
- 130 23. The compound of Claim 22 wherein the pro-

- line ring is in the L - configuration; when R₂ is other than hydrogen the asymmetric carbon to which R₂ is attached is in the D - configuration; and the - O - R₁ group is at the 4-position of the proline ring.
- 5 24. The compound of Claim 23, (cis) - 4 - (4 - fluorophenoxy) - 1 - (D - 3 - mercapto - 2 - methyl - 1 - oxopropyl) - L - proline.
25. The compound of Claim 6 wherein X is sulfur.
26. The compound of Claim 25 wherein R₄ is hydro-
10 gen; R₂ is hydrogen or methyl; and n is one.
27. The compound of Claim 26 wherein R₁ is lower alkyl of 1 to 3 carbons.
28. The compound of Claim 27 wherein R₁ is methyl.
- 15 29. The compound of Claim 28, (trans) - 1 - (3 - mercapto - 1 - oxopropyl) - 3 - methylthio - D,L - proline.
30. The compound of Claim 26 wherein R₁ is



R₆ is hydrogen, methyl, methoxy, methylthio, chloro, fluoro, trifluoromethyl or hydroxy; and p is zero, one or two.

31. The compound of Claim 30, 1 - (D - 3 - mercapto - 2 - methyl - 1 - oxopropyl) - cis - 4 - phenylthio - L - proline.
32. The compound of Claim 7 wherein R₄ is acetyl; R₂ is hydrogen or methyl; and n is one.
33. The compound of Claim 32 wherein R₁ is lower alkyl of 1 to 3 carbons.
34. The compound of Claim 33 wherein the proline ring is in the L - configuration; when R₂ is other than hydrogen the asymmetric carbon to which R₂ is attached is in the D - configuration; and - O - R₁ group is at the 4 - position of the proline ring and in the cis configuration.
35. The compound of Claim 34 wherein R₁ is methyl.
36. The compound of Claim 35, (cis) - 4 - methoxy - 1 - [D - (acetylthio) - 2 - methyl - 1 - oxopropyl] - L - proline.
37. The compound of Claim 33 wherein the proline ring is in the L - configuration; when R₂ is other than hydrogen the asymmetric carbon to which R₂ is attached is in the D - configuration; and the - O - R₁ group is at the 4-position of the proline ring and in the trans configuration.
- 50 38. The compound of Claim 37 wherein R₁ is methyl.
39. The compound of Claim 37 wherein R₁ is ethyl.
40. The compound of Claim 37 wherein R₁ is n - propyl.
41. The compound of Claim 32 wherein R₁ is

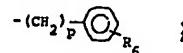


60 R₆ is hydrogen, methyl, methoxy, methylthio, chloro, fluoro, trifluoromethyl or hydroxy; and p is zero, one or two.

42. The compound of Claim 25 wherein R₄ is acetyl; R₂ is hydrogen or methyl; and n is one.
- 65 43. The compound of Claim 42 wherein R₁ is

lower alkyl of 1 to 3 carbons.

44. The compound of Claim 42 wherein R₁ is



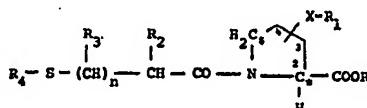
R₆ is hydrogen, methyl, methoxy, methylthio, chloro, fluoro, trifluoromethyl or hydroxy; and p is zero, one or two.

- 75 45. A pharmaceutical composition comprising an effective amount of a compound of any preceding claim and a pharmaceutically acceptable carrier therefor.

- 80 46. A method for reducing blood pressure in hypertensive mammals which comprises administering an effective amount of the composition of Claim 45.

47. A process for preparing a compound of the formula

85



- 90 wherein the X - R₁ group is located at the 3 - or 4 - position of the proline ring;

X is oxygen or sulfur;

R is hydrogen or lower alkyl;

R₁ is lower alkyl, lower alkenyl, lower alkynyl,

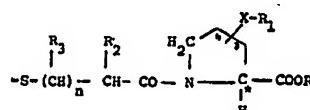
- 95 phenyl, substituted phenyl, phenyl-lower alkylene, or substituted phenyl-lower alkylene;

R₂ and R₃ are independently selected from hydrogen, lower alkyl and trifluoromethyl;

100



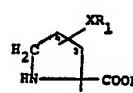
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R₆ is lower alkyl, phenyl, or phenyl - lower alkylene; and

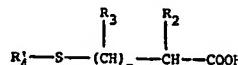
- n is 0, 1 or 2 or such a compound in salt form, comprising coupling a proline compound of the formula

115



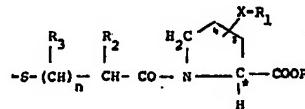
with an acid or its chemical equivalent of the formula

120

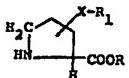


- wherein R_{4'} is hydrogen or R₅ - CO - to form a product wherein R₄ is hydrogen or R₅CO, and if desired, hydrolyzing said product wherein R₄ is R₅CO to form the product wherein R₄ is hydrogen and if desired oxidizing a product wherein R₄ is hydrogen with iodine to form the product wherein R₄ is

130



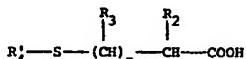
48. A process according to claim 47 wherein a compound of the formula



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is reacted with an acid or its chemical equivalent of the formula

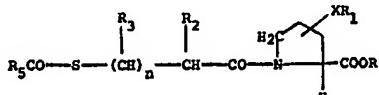
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to form a product wherein R₄ is hydrogen or R₅-CO.

15

49. A process according to claim 47 wherein a compound of the formula

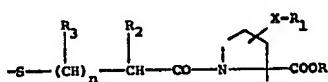


20

is hydrolyzed to form a product wherein R₄ is hydrogen.

25

50. A process according to claim 47 wherein a product wherein R₄ is hydrogen is oxidized with iodine to form a product wherein R₄ is



30

51. The process of any one of claims 47-50 wherein X is oxygen.

52. The process of any one of claims 47-50 wherein X is sulfur.

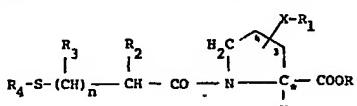
35

53. The process of any one of claims 47-49 wherein R₄ is hydrogen.

54. The process of any one of claims 47-50 wherein the proline ring is in the L-configuration.

40

55. A process according to any one of claims 47-49 wherein the product is of the formula



45

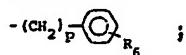
wherein the X-R₁ group is located at the 3- or 4-position of the proline ring;

X is oxygen or sulfur;

R is hydrogen;

50

R₁ is lower alkyl of 1 to 3 carbons or



55

R₂ is hydrogen, methyl or trifluoromethyl;

R₃ is hydrogen;

R₄ is hydrogen, acetyl or benzoyl;

R₆ is hydrogen, methyl, methoxy, methylthio, chloro, fluoro, trifluoromethyl or hydroxy;

n is zero or one; and

60

p is zero, one or two, or such a compound in salt form.

56. The process of claim 55 wherein X is oxygen.

57. The process of claim 56 wherein R₄ is hydrogen; R₂ is hydrogen or methyl; and n is one.

65

58. The process of claim 57 wherein R₁ is lower

alkyl of 1 to 3 carbons.

59. The process of claim 58 wherein the proline ring is in the L-configuration; when R₂ is other than hydrogen the asymmetric carbon to which R₂ is attached is in the D-configuration; and the -O-R₁ group is at the 4-position of the proline ring and in the cis configuration.

60. The process of claim 59 wherein R₁ is methyl.

61. The process of claim 60 wherein the product is (cis)-4-methoxy-1-(D-3-mercaptop-2-methyl-1-oxopropyl)-L-proline.

62. The process of claim 60 wherein the product is (cis)-4-methoxy-1-(3-mercaptop-1-oxopropyl)-L-proline.

63. The process of claim 58 wherein the proline ring is in the L-configuration; when R₂ is other than hydrogen the asymmetric carbon to which R₂ is attached is in the D-configuration; and the -O-R₁ group is at the 4-position of the proline ring and in the trans configuration.

64. The process of claim 63 wherein R₁ is methyl.

65. The process of claim 64 wherein the product is (trans)-4-methoxy-1-(D-3-mercaptop-2-methyl-1-oxopropyl)-L-proline.

66. The process of claim 64 wherein the product is trans-4-methoxy-1-(3-mercaptop-1-oxopropyl)-L-proline.

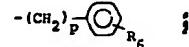
67. The process of claim 63 wherein R₁ is ethyl.

68. The process of claim 67 wherein the product is (trans)-4-ethoxy-1-(D-3-mercaptop-2-methyl-1-oxopropyl)-L-proline.

69. The process of claim 63 wherein R₁ is *n*-propyl.

70. The process of claim 69 wherein the product is (trans)-4-propoxy-1-(D-3-mercaptop-2-methyl-1-oxopropyl)-L-proline.

71. The process of claim 57 wherein R₁ is



105

R₆ is hydrogen, methyl, methoxy, methylthio, chloro, fluoro, trifluoromethyl or hydroxy; and p is zero, one or two.

72. The process of claim 71 wherein the proline ring is in the L-configuration; when R₂ is other than hydrogen the asymmetric carbon to which R₂ is attached is in the D-configuration; and the -O-R₁ group is at the 4-position of the proline ring.

73. The process of claim 72 wherein the product is (cis)-4-(4-fluorophenoxy)-1-(D-3-mercaptop-2-methyl-1-oxopropyl)-L-proline.

74. The process of claim 55 wherein X is sulfur.

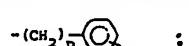
75. The process of claim 74 wherein R₄ is hydrogen; R₂ is hydrogen or methyl; and n is one.

76. The process of claim 75 wherein R₁ is lower alkyl of 1 to 3 carbons.

77. The process of claim 76 wherein R₁ is methyl.

78. The process of claim 77 wherein the product is (trans)-1-(3-mercaptop-1-oxopropyl)-3-methylthio-D,L-proline.

79. The process of claim 75 wherein R₁ is



R₆ is hydrogen, methyl, methoxy, methylthio, chloro, fluoro, trifluoromethyl or hydroxy; and p is zero, one or two.

80. The process of claim 79 wherein the product
5 is 1 - (D - 3 - mercapto - 2 - methyl - 1 - oxopropyl) -
cis - 4 - phenylthio - L - proline.

81. The process of claim 56 wherein R₄ is acetyl;
R₂ is hydrogen or methyl; and n is one.

82. The process of claim 81 wherein R₁ is lower
10 alkyl of 1 to 3 carbons.

83. The process of claim 82 wherein the proline
ring is in the L - configuration; when R₂ is other than
hydrogen the asymmetric carbon to which R₂ is
attached is in the D - configuration; and - O - R₁
15 group is at the 4-position of the proline ring and in
the cis configuration.

84. The process of claim 83 wherein R₁ is methyl.

85. The process of claim 84 wherein the product
is (cis) - 4 - methoxy - 1 - [D - (acetylthio) - 2 - methyl -
20 1 - oxopropyl] - L - proline.

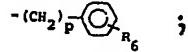
86. The process of claim 89 wherein the proline
ring is in the L - configuration; when R₂ is other than
hydrogen the asymmetric carbon to which R₂ is
attached is in the D - configuration; and the - O - R₁
25 group is at the 4-position of the proline ring and in
the trans configuration.

87. The process of claim 86 wherein R₁ is methyl.

88. The process of claim 86 wherein R₁ is ethyl.

89. The process of claim 86 wherein R₁ is n -
30 propyl.

90. The process of claim 81 wherein R₁ is

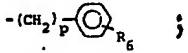


35 R₆ is hydrogen, methyl, methoxy, methylthio, chloro, fluoro, trifluoromethyl or hydroxy; and p is zero, one or two.

91. The process of claim 74 wherein R₄ is acetyl;
R₂ is hydrogen or methyl; and n is one.

40 92. The process of claim 91 wherein R₁ is lower
alkyl of 1 to 3 carbons.

93. The process of claim 91 wherein R₁ is



45 R₆ is hydrogen, methyl, methoxy, methylthio, chloro, fluoro, trifluoromethyl or hydroxy; and p is zero, one or two.

94. A compound according to claim 1 when prepared by a process according to any one of claims
50 47-93.

95. A compound according to claim 1, as named in any of the Examples.

96. A compound according to any one of claims
55 1-44, 94 and 95 for use as a hypotensive agent.

97. A composition according to claim 43 in the form of a tablet, capsule or sterile injectable preparation.

98. A composition according to claim 43 or 97,
60 including a binder, preservative, stabilizer, excipient, disintegrating agent, lubricant, sweetening agent, or flavouring agent.